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Information in Support of a Recovery Potential Assessment of Purple Wartyback (*Cyclonaias tuberculata*) in Canada

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

The Purple Wartyback (*Cyclonaias tuberculata*) is a long-lived species of freshwater mussel currently found in three watersheds in Canada from lower Lake Huron through Lake St. Clair. The species was assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in May 2021 as Threatened, owing to a small number of extant locations and a continuing decline of habitat quality throughout its range. The species is considered extirpated from two historical locations. The Recovery Potential Assessment provides background information and scientific advice needed to fulfill various requirements of the *Species at Risk Act* (SARA). This research document provides the current state of knowledge on the species including its biology, distribution, population trends, habitat requirements, and threats. Purple Wartyback is a short-term brooder that is thought to use North American catfishes (Ictaluridae) as hosts for completing its life cycle. It is found in relatively deep, medium to large rivers with moderate to swift currents and occasionally lentic areas over sand, gravel, and cobble substrates. Long-term standardized sampling data suggest that at least two of the three populations in Canada may be growing; however, more years of data are required to span a full generation of this long-lived species. A threat assessment identified the greatest threats to Purple Wartyback in Canada as pollution from agricultural and urban sources, climate change (notably droughts), aquatic invasive species (including dreissenid mussels and Round Goby (*Neogobius melanostomus*)), and dredging. Limited information exists evaluating the impacts of these threats on Purple Wartyback specifically. Mitigation measures and alternative activities regarding habitat-related threats are presented. Important knowledge gaps remain surrounding the full extent of its distribution within known watersheds, habitat preferences by life stage, mussel-host interactions, and physiological tolerances to environmental conditions and pollutants.

INTRODUCTION

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assessed the status of Purple Wartyback (*Cyclonaias tuberculata*) in May 2021 as Threatened (COSEWIC 2021). The reason for this designation was that the species has a small and restricted range, is known from only three rivers in Ontario (Ausable, Sydenham, and Thames rivers), and is considered extirpated from two historically occupied areas (Detroit River and western Lake Erie). Additionally, the habitat quality through its range is declining due to agricultural and urban sources of pollution, impacts from climate change (droughts), aquatic invasive species (AIS), and dredging activities. Purple Wartyback is a long-lived species, and like all freshwater mussels, is sedentary at the juvenile and adult stages making it particularly vulnerable to habitat-related threats, and as an obligate parasite at the larval stage, it may be limited by its host fishes as well. Purple Wartyback is not currently listed under the *Species at Risk Act* (SARA).

Fisheries and Oceans Canada (DFO) developed the recovery potential assessment (RPA) process to provide information and science-based advice needed to inform listing decisions and fulfill requirements of SARA, including the development of recovery strategies and authorizations to carry out activities that would otherwise violate SARA. The process is based on DFO (2007) and updated guidelines (DFO unpublished) that assess 22 recovery potential elements. This document summarizes information about the biology, distribution, population parameters, and threats and applicable mitigation measures to support the RPA process for Purple Wartyback. This research document accompanies a recovery potential modeling research document (van der Lee and Koops 2023) and together these address the 22 elements outlined in the RPA process (DFO 2007, DFO unpublished).

BIOLOGY, ABUNDANCE, DISTRIBUTION AND LIFE HISTORY PARAMETERS

Element 1: *Summarize the biology of Purple Wartyback.*

DESCRIPTION

Purple Wartyback has a thick shell that is typically rounded on the anterior side and squared-off on the posterior side with a wing behind the beak (Metcalfe-Smith et al. 2005, Sietman 2018, WIDNR 2020, COSEWIC 2021, MSU 2022). The beaks are low, the beak cavity deep, and the beak sculpture has numerous wavy (or zig-zag) ridges. The shell is compressed with numerous nodules of variable shape that may turn into ridges on the wing. The pseudocardinal teeth are very large and serrated, and the lateral teeth are short and often slightly curved. The shell morphology of Purple Wartyback (i.e., the presence of nodules, beak sculpturing, low/compressed profile, and the squared-off dorsal wing) suggest it is adapted to withstand high flows (Watters 1994). The periostracum is typically yellow-brown to red-brown in adults, but generally yellowish in juveniles. The nacre is purple and iridescent (Metcalfe-Smith et al. 2005). The species is often reported to reach 130 mm in length, but can reach a maximum of 200 mm in Canada (COSEWIC 2021). It is not sexually dimorphic. Purple Wartyback is considered one of the easier freshwater mussel species to identify, and can be distinguished from the closely-related and spatially overlapping species Pimpleback (*Cyclonaias pustolusa*), which has a broad green ray on the beak and nodules that do not extend up onto the beak, and the Mapleleaf (*Quadrula quadrula*), which is more quadrate and typically has nodules that extend in two distinct lines from the beak to the ventral margin on either side of a central sulcus. Mapleleaf typically have a white nacre (Sietman 2018, Toronto Zoo 2021).

LIFE CYCLE

Purple Wartyback is a dioecious species, and cases of hermaphrodism are rare (Haggerty et al. 1995, Boyles 2004). Male and female Purple Wartyback show evidence of gamete production throughout the year, with mature gametes typically ready in November and held overwinter. Spawning occurred in early spring through summer in West Virginia and Tennessee rivers (Jirka and Neves 1992, Haggerty et al. 1995, COSEWIC 2021). Like all freshwater mussels, males release sperm through their excurrent siphon, and it is filtered through the gills of females located downstream. Reproductive success may be partly influenced by river discharge, as it can dictate the distribution of sperm available to females (Haggerty et al. 1995). Males start to release sperm as early as March when water temperatures reach approximately 9°C, and may continue through to July (Jirka and Neves 1992, Haggerty et al. 1995). Females begin spawning (i.e., ova no longer present in alveolar lumina) in spring when water temperatures reached approximately 10°C, typically early April through June (Jirka and Neves 1992).

Once filtered by the female, the sperm enters the posterior portion of the gill (suprabranchial chambers) where mature ova are stored and then fertilized, and embryos mature in the outer set of gills (marsupia). This differs from other members of the Quadrulini tribe that use all four sets of gills for brooding (Campbell et al. 2005). Purple Wartyback is a short-term brooder (tachytictic), meaning that eggs are fertilized and glochidia released within the same spawning season. From a study in the Tennessee River, 75% of female Purple Wartyback examined had embryos in the suprabranchial chambers in early April, most females had embryos in the outer gills by late May through mid-July, and mature glochidia were found mostly in late July through August (Haggerty et al. 1995). In a West Virginia river, mature glochidia were first reported in females in May, and timing of glochidia release appeared to correlate with warming water temperatures; no glochidia were released ≤ 9°C (Jirka and Neves 1992). Abundance of Purple Wartyback glochidia in the Sydenham River, Ontario was highest in late summer compared to early fall (Smodis 2022). Purple Wartyback glochidia are easily identifiable; they are relatively large, with a smooth, rounded edge and no hooks (Jirka and Neves 1992, Tremblay et al. 2015). Glochidia collected from gravid females in the Ausable, Sydenham and Thames rivers had a mean shell length of 0.264 mm (±0.005 mm SD), mean shell height of 0.325 mm (±0.009 mm SD), and mean hinge length of 0.124 mm (±0.005 mm SD) (Tremblay et al. 2015), and Watters et al. (2009) reports mean lengths from U.S. specimens of 0.267 mm (range: 0.25–0.29 mm) and mean heights of 0.325 mm (range: 0.28–0.35 mm).

Once mature, the glochidia must encounter a host fish on which they encyst for completing their development. It is believed that Purple Wartyback use larger members of the North American catfishes family (Ictaluridae), including Black and Yellow bullheads (*Ameiurus melas, A. natalis*), Channel Catfish (*Ictalurus punctatus*) and Flathead Catfish (*Pylodictis olivaris*) based on laboratory infestation studies from the U.S. (Hove et al. 1994, Hove et al. 1997). Female freshwater mussels use a variety of host attraction techniques to increase the chances of glochidial encounters with and infestation on the host. Two host attraction techniques have been observed in female Purple Wartyback in U.S. populations, a mantle display and amorphous conglutinates; however, females appear to use one or the other in a given spawning season (Sietman et al. 2012). If a mantle display was used, glochidia were present in the stomate-shaped, mantle magazine (inflated tissue around the excurrent siphon). If a conglutinate was made, they formed loose, gelatinous strands of mucous with embedded glochidia. It remains unclear whether Purple Wartyback exhibit reflexive release of glochidia (Barhnart et al. 2008, Sietman et al. 2012). In Minnesota, female Purple Wartyback displayed their mantles from June through early August when water temperatures ranged from 19–27°C, with individuals displaying for approximately one month (Sietman et al. 2012).

Sietman et al. (2012) described the appearance of Purple Wartyback conglutinates as pale in colour and resembling dead tissue. As ictalurid hosts are omnivorous, nocturnal, and benthic feeding, consuming plant material, insects, molluscs and fish (Scott and Crossman 1998), these non-specific conglutinates are likely effective for attracting these hosts. Using a 24-hour glochidia auto-sampler in the Sydenham River, Smodis (2022) detected Purple Wartyback glochidia throughout the sampling period, but abundance was highest at dawn and dusk, the latter peak suggests timing of release is generally well matched with periods of activity for presumed catfish hosts. There is currently no information available regarding host encounter, infestation, metamorphosis, or survival rates for Purple Wartyback glochidia as for some other mussel species (McNichols et al. 2011). Ideally, host fish are present in sufficient numbers and are of good health to act as a candidate host (Bouvier et al. 2014), but species that display glochidia with lures or conglutinates are thought to be less dependent on host density (Haag and Warren 1998). Periods of encystment for Purple Wartyback glochidia have been reported to be 17–38 days, but timing may depend on water temperature and species of host (Hove et al. 1994, 1997). During encystment, glochidia feed on the body fluids of the host and undergo a metamorphosis. Additionally, dispersal of the host fish allows for upstream movement of mussels and genetic exchange between subpopulations.

After the period of encystment, the juvenile mussels drop off the host fish and burrow into the sediment where they remain for several years (likely > 6 years for Purple Wartyback) for growth (Jirka and Neves 1992, COSEWIC 2021, van der Lee et al. in prep.[1]). Once mature, adult mussels move up to the sediment surface and remain relatively sedentary. Adults make vertical movements within the upper layer of the substrate (10-15 cm) seasonally (i.e., during winter), with changes in water level or temperature, and body size may also influence propensity for vertical movements (Schwalb and Pusch 2007, Sullivan and Woolnough 2021). Mussels may also make short-distance horizontal movements daily and seasonally in response to changing water levels or other adverse conditions, during spawning season, and sometimes erratically, possibly for feeding (Balfour and Smock 1995, Schwalb and Pusch 2007). Observed horizontal movements varied with species and depth/flow conditions, but have been reported to range from a mean of 2 cm/day (range 0–32 cm/day) for species examined in German streams (Schwalb and Pusch 2007), and up to a mean of 14 cm/day (range 0–128 cm/day) for Snuffbox (*Epioblasma triquetra*) in Michigan streams (Sullivan and Woolnough 2021) and a mean of 57 cm/day (range 1–141 cm/d) for Fat Pocketbook (*Potamilus capax*) in Arkansas (Peck et al. 2014).

Purple Wartyback is a long-lived species (Haag 2012). Recent aging data suggests it may live to over 90 years (van der Lee et al. in prep.[1]), and the generation time of Canadian populations was estimated to be 26 years from stochastic projection matrices (van der Lee and Koops 2023). The youngest mature female reported from New River, West Virginia was age 6 (56.8 mm in length; Jirka and Neves 1992), and age at maturity in Canada is estimated to be 7.2 years (53.14 mm in length) from a predictive relationship with the von Bertalanffy growth coefficient k (van der Lee et al. in prep.[1]). Length distribution data from the three Canadian populations indicate that recruitment has likely occurred recently in all three (COSEWIC 2021, van der Lee et al. in prep.[1]). In the Ausable River, recruitment was observed at the same 3 of 8 sites in all three sampling periods (2006–2008, 2011–2013, 2018–2022) and at one additional site in the most recent sampling period; juveniles made up a mean of 33.8% of individuals at

[1] van der Lee, A.S., Goguen, M.N., McNichols-O'Rourke, K.A., Morris, T.J., and Koops, M.A. In prep. Evaluating the status and biology of an imperilled freshwater mussel, Purple Wartyback (*Cyclonaias tuberculata*), in Southern Ontario. In preparation.

quadrat sites. In the Sydenham River, recruitment was observed in follow up monitoring (2012–2018) at 6 of 7 sites where it was observed during initial surveys (1999–2003), and juveniles made up a mean of 13.2% of individuals at quadrat sites. Similarly, in the Thames River, recruitment was observed at 3 of 7 sites during follow-up monitoring (2015–2017) compared to 0 sites from initial surveys (2004–2010), and juveniles made up a mean of 46.8% of individuals at quadrat sites (COSEWIC 2021, van der Lee et al. in prep.[1]).

From quadrat surveys from 1997 through 2021, the mean shell length of Purple Wartyback detected in the Ausable River was 60.3 mm, 80.4 mm in the Sydenham River, and 59.8 mm in the Thames River (van der Lee et al. in prep.[1]). The largest Canadian specimen was 198.9 mm in length observed in the Sydenham River, and the oldest individual was aged 92 years (van der Lee et al. in prep.[1]; Figure 1). Annual length-frequency distributions from quadrat surveys in the Ausable, Sydenham, and Thames rivers are presented in Figure 2.

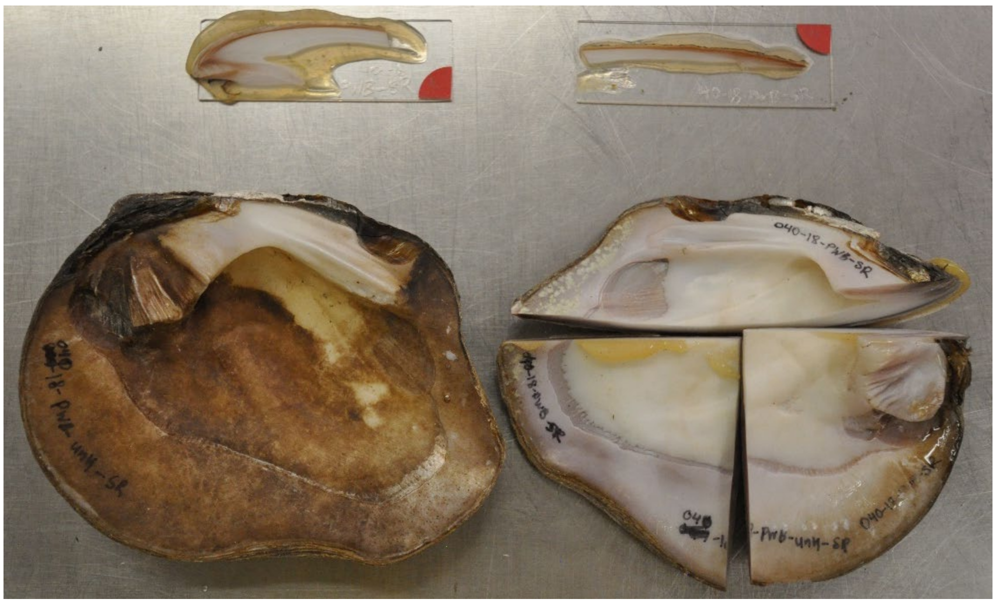

Figure 1. A Purple Wartyback collected from the Sydenham River in 2018 measuring 128.04 mm in length. The age was estimated to be 92 years. Photo Credit: Rachel Jones (DFO).

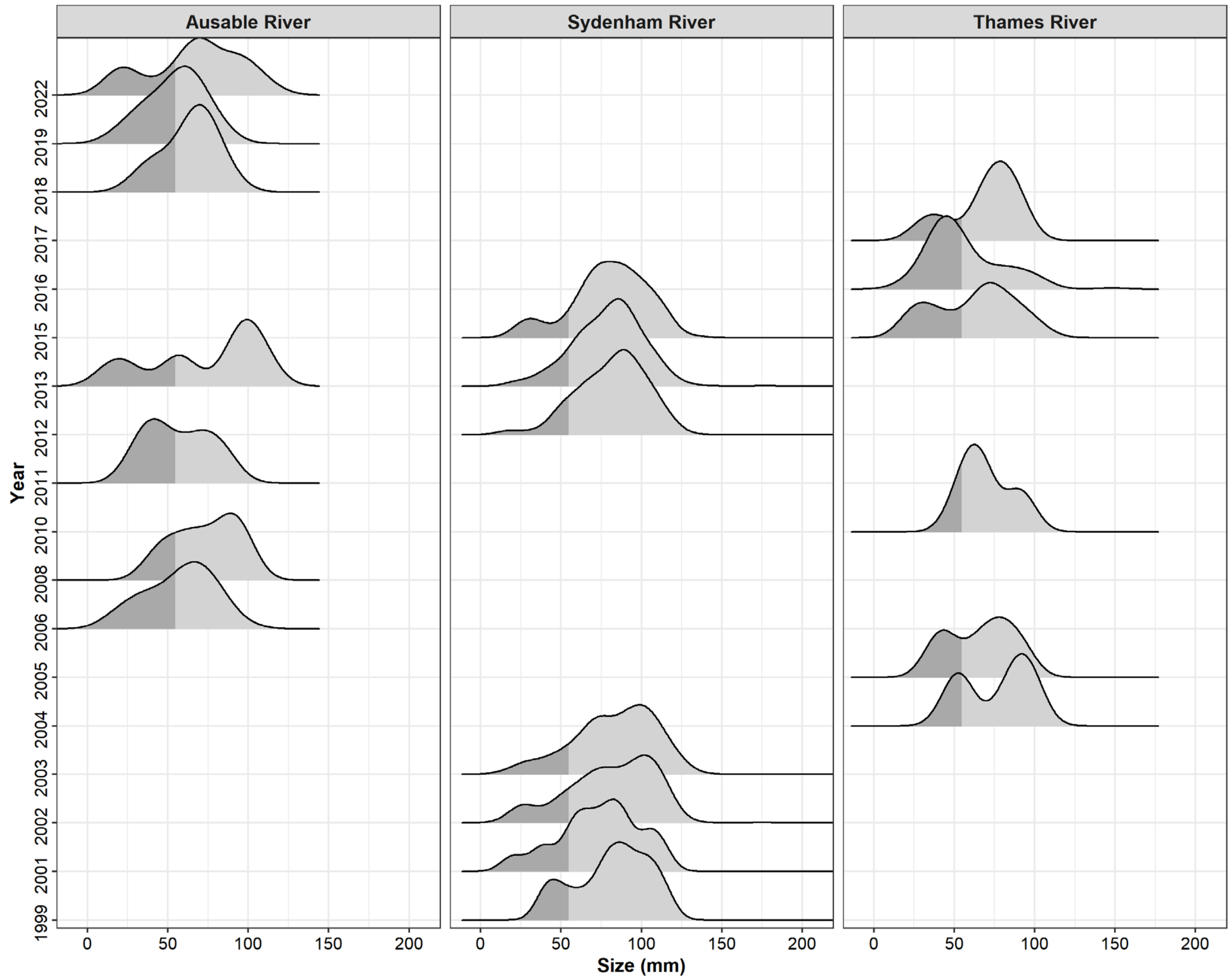

Figure 2. Length-frequency distribution by year for the Ausable, Sydenham, and Thames rivers from quadrat samples. Dark grey shading represents individuals < 53.1 mm in length, presumed to be juveniles, indicating recruitment has occurred in recent years. Figure modified from van der Lee et al. (in prep.[1]) to include the Ausable River. The sample sizes differed among populations (Ausable: n=136; Sydenham: n=3085; and Thames: n=190), thus the scales are not directly comparable between watersheds.

FEEDING AND DIET

Adult unionid mussels are suspension feeders, generally consuming organic debris, algae and bacteria from the water column and sediment. Currents are created around the mussel by cilia to circulate new, unfiltered water around the incurrent siphon. Material is sorted on the palp, food particles are directed towards the mouth for digestion, and other material is packaged in mucous and expelled as pseudofeces. Juveniles remain buried in the sediment for the first few years of life and feed on organic material available through interstitial pore water. Pedal feeding is often employed by newly metamorphosed juveniles, where the foot moves along the substrate to collect particles, is drawn back in, and cilia on the foot direct food particles towards the mouth. The length of the pedal-feeding stage appears variable by species (Gatenby et al. 1997). Larval mussels (glochidia) feed on host fish tissue while encysted. There have been no diet studies on Purple Wartyback, but it is thought that different species may preferentially select specific food items and/or specific particle sizes, which may reduce competition in diverse mussel assemblages (Beck and Neves 2003, Tran and Ackerman 2019).

SPECIAL SIGNIFICANCE

Freshwater mussels, in general, are good indicators of water quality and stream health as they are long-term inhabitants of aquatic ecosystems, relatively immobile, and pollution sensitive. Additionally, as filter-feeders, they aid in numerous water, nutrient and sediment cycling processes (COSEWIC 2021). Mussels are often preyed upon by terrestrial mammals (e.g., muskrat and raccoon) and may aid in energy transfer to terrestrial systems (Neves and Odom 1989).

Element 2: *Evaluate the recent species trajectory for abundance, distribution and number of populations.*

ABUNDANCE

Reliable abundance estimates are lacking for all populations of Purple Wartyback in Canada. To coarsely understand relative population size, COSEWIC (2021) calculated catch per unit effort (CPUE; from timed-search surveys) and average density (estimated from quadrat surveys)[2] for each river; additionally, the occupied reach length in each river is approximated based on length of continuous Ontario Hydro Network stream segments with occurrence records (Mandrak et al. 2014) (Table 1). Site-specific abundance estimates were generated using quadrat survey data from DFO's Unionid Monitoring and Biodiversity Observation (UMBO) monitoring network for the Sydenham and Thames rivers (van der Lee et al. in prep.[1]), and for the Ausable River following methods in van der Lee et al. (in prep.[1]) (additional model details provided in Appendix A). A hierarchical Bayesian model was used to project site-specific density estimates across the entirety of surveyed habitat. Projections were made for 2022 in the Ausable River, 2015 in the Sydenham River, and 2017 for the Thames River; these years represent the most recent year of sampling in each river. This yielded abundance estimates of 294 (95% credible intervals (CI): 207–409) Purple Wartyback in the Ausable River, 10504 (95% CI: 9563–11505) in the Sydenham River, and 872 (95% CI: 696–1091) in the Thames River, covering approximately 2490 m^2, 3600 m^2, 3000 m^2, respectively, in each river (Appendix A, van der Lee et al. in prep.[1]). Population growth rates were also estimated from this model using quadrat data from 2006–2022 in the Ausable River, 1999–2015 from the Sydenham River and 2004–2017 from the Thames River with populations in the latter two rivers having increased in size since the survey commenced and in the Ausable River a significant trend was not detected (Table 1).

[2] COSEWIC 2021 also coarsely estimated population abundance by extrapolating the mean site-level densities across the known distribution in each river. The population estimates likely overestimate the true population size, as sampling was designed for evaluating trends through time and not for estimating population size, so are not included here.

Table 1. Current catch per unit effort (CPUE; individuals/Person-Hour) from timed-search surveys, and mean density from quadrat surveys for Purple Wartyback in Canada; adapted from COSEWIC (2021). An estimate of occupied habitat is provided based on continuous, occupied Ontario Hydro Network segments. Median density and population growth rate estimates (including 95% credible intervals (CI)) from van der Lee et al. (in prep.[1]) for the Sydenham and Thames rivers, and calculated for the Ausable River following methods in van der Lee et al. (in prep.[1]).

Locality	CPUE (ind/PH ± SE)	Mean Density (live/m^2 ± SE)	Approximate occupied river length (km)	Median Density (live/m^2) (95% CI)	Population Growth Rate (95% CI)
Ausable River	0.61 (± 0.17)	0.09 (± 0.03)	62.2 km	0.031 (CI: 0.002-0.25)	1.016 (CI: 0.985-1.049
Sydenham River	6.63 (± 2.38)	2.52 (± 0.76)	85.9 km	1.82 (CI: 0.94-3.87)	1.047 (CI: 1.037-1.058)
Thames River	1.53 (± 0.27)	0.26 (± 0.12)	136.0 km (lower) 23.6 km (South) 9.6 km (North)	0.12 (CI: 0.03-0.42)	1.157 (CI: 1.10-1.221)

DISTRIBUTION

Globally, Purple Wartyback is known from the Mississippi River and lower Great Lakes drainages. It is found in the province of Ontario in Canada, and in 18 states in the U.S.A., including: Alabama, Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Mississippi, Missouri, North Carolina, Ohio, Oklahoma, Tennessee, Virginia, West Virginia, and Wisconsin. It is thought to be extirpated from Pennsylvania and South Dakota (Woolnough and Bogan 2017).

CURRENT STATUS

In Canada, the current and historical distribution of Purple Wartyback is limited to five waterbodies in southwestern Ontario, two of which are currently considered extirpated (Figure 3, Appendix B). Extant locations include the Ausable River in the Lake Huron drainage, and the Sydenham and Thames rivers (including North Thames River, South Thames River, and main stem) in the Lake St. Clair drainage. Purple Wartyback is thought to be extirpated from the Detroit River and Lake Erie around Pelee Island. Since its discovery in Canada, there have been approximately 7000 live individuals observed from over 200 sampling records (DFO Lower Great Lakes Unionid Database (LGLUD) unpublished data, COSEWIC 2021). Sampling efforts have been a combination of timed-search surveys and standardized quadrat (UMBO) surveys. Timed-search surveys are generally used for broadly understanding species distributions. They use a combination of visual and tactile methods, which can cover a large area relatively quickly but tend to bias towards larger individuals. UMBO quadrat surveys are designed for evaluating trends through time and population demographics. They invoke a stratified random design with 20% coverage whereby a 400 m^2 site is divided into 15 m^2 blocks and three 1 m^2 quadrats are excavated to 15 cm below the substrate surface within each block Although sampling information (time or area of search effort, sampling method) and specimen condition or even number collected were seldom reported with historical records, it is summarized for current records (1997–2021) in Appendix B.

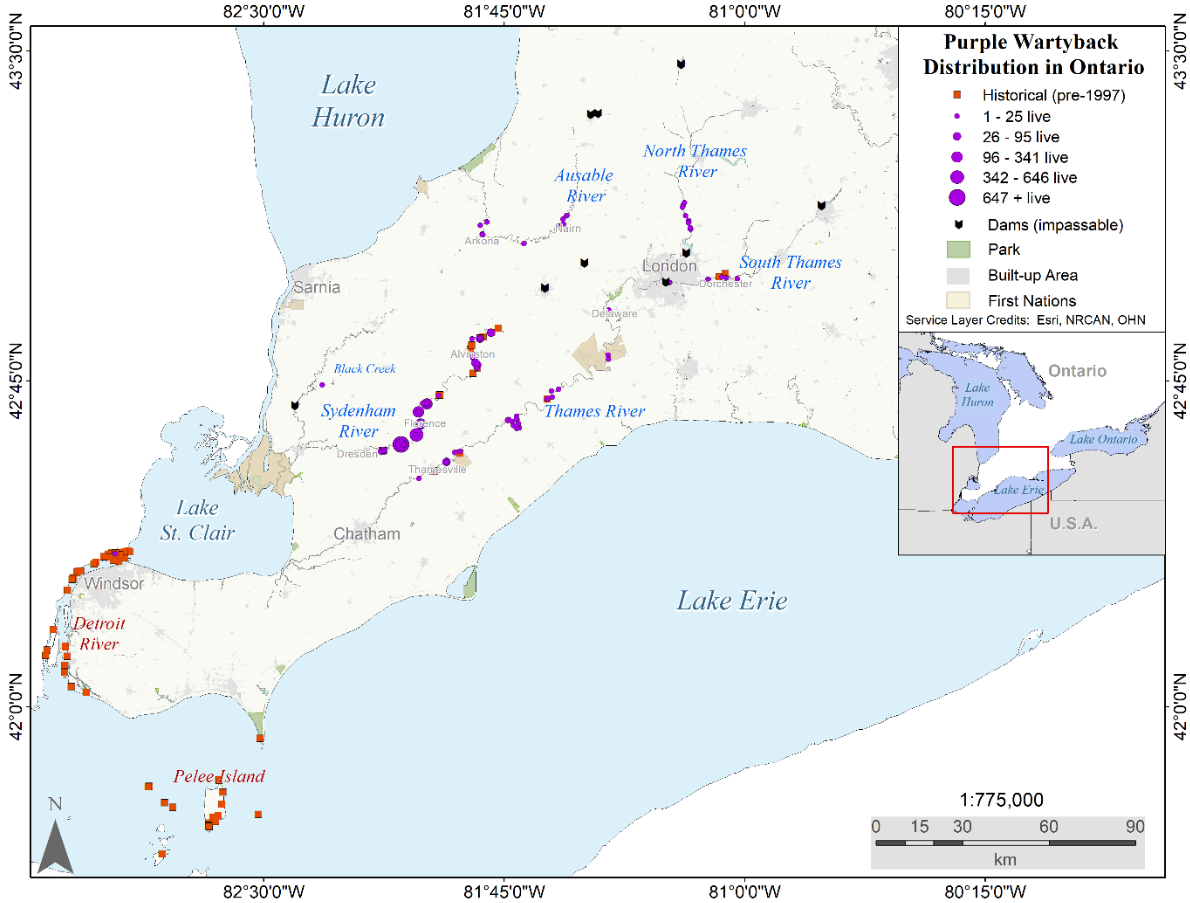

Figure 3. Distribution of Purple Wartyback in Canada. Red squares are historical records (prior to 1997) and may represent live individuals, or shells of variable or unknown condition. Purple circles indicate recent (1997–2021) collections of live individuals only.

Ausable River

The occurrence of Purple Wartyback in the Ausable River is a relatively new discovery, likely the result of increased search effort. The Ausable River is the only river in the Lake Huron drainage known to support the species. The species was first reported in 1998 when four live individuals and two fresh shells (one whole, one valve) were detected during a timed-search survey. The species was subsequently detected during timed-search surveys in 2002 (n=2), 2004 (1 weathered shell), 2007 (n=2), 2008 (n=5 + 1 weathered shell), 2012 (n=25), 2013 (n=26), 2014 (n=3), 2015 (n=2), 2016 (n=10), and 2018 (n=1). The species was detected during quadrat surveys in 2006 (n=38; 506 m^2), 2008 (n=9; 75 m^2), 2011 (n=26; 534 m^2), 2013 (n=9; 75 m^2), 2018 (n=12; 301 m^2), 2019 (n=27; 226 m^2), and 2022 (n=15; 75 m^2). The distribution of live animals in the Ausable River was previously thought to be two distinct stretches of river, one at Nairn and one at Arkona, totaling approximately 18.5 km (COSEWIC 2021); however, a detection in 2019 in between these reaches suggest Purple Wartyback may occupy a longer stretch, approximately 62.2 km^2.

Sydenham River

The Sydenham River is the most diverse system in Canada for freshwater mussels, supporting 34 species, 13 of which are SARA-listed or COSEWIC-assessed as at risk (McNichols-O'Rourke et al. 2012, LGLUD unpublished data). Purple Wartyback was first documented in the East Sydenham River in 1963 (n=5), representing the first live individuals of the species reported in Canada. Live individuals have been consistently detected since that time, with records from 1965 (n=3 + 25 fresh shells), 1967 (n=10), 1971 (n=17), 1973 (n=14 + 8 fresh shells), 1985 (n=1), 1991 (n=21), 1997 (n=241 + 53 fresh shells and 1 fresh valve), 1998 (n=40 + 3 fresh shells), 2002 (n=45), 2008 (n=110), 2010 (n=25), 2012 (n=51), 2013 (n=74), 2014 (n=153), 2015 (n=50), 2017 (n=192), 2018 (n=29), 2019 (n=11), 2020 (n=47), and 2022 (n=110) representing timed-search surveys. Quadrat surveys also detected Purple Wartyback in 1999 (n=44; 147 m^2 surveyed), 2001 (n=95; 230 m^2), 2002 (n=659; 381 m^2), 2003 (n=392; 387 m^2), 2012 (n=2835; 669 m^2), 2013 (n=907; 375 m^2), 2015 (n=374; 225 m^2), 2017 (n=25; 50 m^2), 2020 (n=221; 417 m^2), 2021 (n=265; 80 m^2), and 2022 (n=570; 375 m^2). In 2022, the Ontario Ministry of Natural Resources and Forestry (MNRF) conducted brail surveys and detected a single live Purple Wartyback and 2 fresh shells (LeBaron et al. 2023). The distribution of Purple Wartyback in the East Sydenham River is an approximately 85.9 km (nearly continuous) stretch from Napier to downstream of Dresden.

In 2013, a single live Purple Wartyback was incidentally observed in Black Creek, a tributary of the North Sydenham River (COSEWIC 2021). This represents the only occurrence of the species outside of the East Sydenham River in this watershed. Additional individuals representing multiple age and/or size classes captured through time would be needed to confirm whether this represents a population.

Thames River

Evidence of Purple Wartyback was first found in the lower Thames River in 1935 (four fresh shells), and 1965 (one fresh shell), but the first live individual was not detected until 1985. Additional observations of the species were reported from timed-search surveys in 1986 (n=1), 1994 (one fresh valve), 1997 (n=30 + 11 fresh shells and valves, 23 weathered shells and valves), 2005 (n=59 + 1 fresh shell), 2015 (1 weathered shell), 2018 (n=3), 2021 (n=28 + 1 weathered valve), and 2022 (n=10 +1 weathered shell and 1 weathered valve). Purple Wartyback was also detected during quadrat surveys in the Thames River in 2004 (n=9; 336 m^2 surveyed), 2005 (n=6; 75 m^2), 2010 (n=7; 318 m^2), 2012 (n=39; 696 m^2), 2013 (n=37 + 1 weathered shell; 636 m^2), 2015 (n=24; 150 m^2), 2016 (n=125; 375 m^2), 2017 (n=1; 150 m^2). In the lower Thames River, Purple Wartyback is widespread from Delaware to downstream of Thamesville (Kent Bridge), totaling approximately 136 km of river length, but given continuous suitable habitat and a lack of surveys further downstream, the distribution may continue to the mouth of the river, for an additional 46.9 km. In 2022, MNRF conducted brail surveys from Kent Bridge to the confluence with Jeannette's Creek and did not detect Purple Wartyback; preliminary habitat data suggest substrates are softer and habitat may become less suitable moving towards the mouth (LeBaron et al. 2023).

Although Purple Wartyback shells had historically been found in the upper Thames River watershed (above the confluence of the North, South and Middle Thames rivers), the discovery of live specimens is relatively recent. A fresh shell was first observed in the South Thames River in Dorchester in 1936. Only four surveys were undertaken in the upper Thames River watershed until 1997, when the first live individuals (n=2) were found near the historical Dorchester shell record during a timed-search survey. Live individuals were observed in the South Thames River during quadrat surveys in 2004 (n=3; 75 m^2), 2017 (n=8; 75 m^2), and 2018 (n=5; 75 m^2). Weathered shells and valves were observed during timed-searches in 2001, 2003, 2004 and

2015. The distribution of Purple Wartyback in the South Thames River is from Dorchester to within the city of London (upstream of Hunt Dam and the Forks), comprising approximately 21.4 km. In the North Thames River, live individuals were first observed in 2004 (n=9), and subsequently 2008 (n=20), 2021 (n=14), and 2022 (n=72) during timed-searches. Quadrat surveys also detected the species in 2015 (n=6; 75 m^2) and 2018 (n=7; 75 m^2). Observations in the North Thames River occur over a 7 km stretch from Plover Mills to immediately upstream of Fanshawe Lake.

Detroit River

The earliest known record of Purple Wartyback in Canada came from the Detroit River in 1934, although the condition of the specimen is unknown. The first live individuals recorded from the Detroit River were observed in 1982 (n=5 + 4 fresh valves). Purple Wartyback was historically distributed throughout the Detroit River, with most detections from the inlet at Lake St. Clair to the north end of Fighting Island, and from the mouth of the Canard River into the outlet at Lake Erie. A total of 32 live individuals were captured in the 80s and 90s, with an additional 38 fresh valves and 22 weathered valves collected over this time. It had not been detected in the Detroit River since 1998 when one live individual was observed. It is believed that the invasion of dreissenid mussels (*Dreissena polymorpha* and *D. rostriformis*) led to its extirpation, and likely all native unionid mussels, in this system (Schloesser and Nalepa 1994, Schloesser et al. 2006). In 2019, 72 weathered shells were reported from the Detroit River following 29 person-hours of searching from 23 sites; however, no live individuals were collected and the species is considered extirpated from this system (Keretz et al. 2021, COSEWIC 2021).

Lake Erie

Purple Wartyback was historically known from Pelee Island and surrounding islands (East Sister Island, Little Chicken Reef, Hen Island) and Point Pelee National Park. These areas are challenging to sample for mussels due to access and depth. It was first reported there in 1960[3] when 20 fresh shells were observed (19 whole shells, one valve). Since that time, only six live individuals have been reported (1969, n=2; 1970, n=3; 1982, n=1). An additional 236 fresh shells were observed (plus an additional seven fresh valves) from numerous timed-searches from 1961 through 1990. A live individual was last observed in Lake Erie in 1982, and the most recent evidence of the species from this location is five weathered valves collected in 2005. Purple Wartyback is considered extirpated from this location (COSEWIC 2021).

POPULATION ASSESSMENT

To assess the Population Status of Purple Wartyback in Canada, each population was ranked in terms of its abundance (Relative Abundance Index) and trajectory (Population Trajectory; Table 2). The Relative Abundance Index (Extirpated, Low, Medium, High, or Unknown) considers the median density estimates along with the coarse estimates of occupied river length found in Table 1. The Sydenham River is the largest and best studied population in Canada, so the other populations are assessed relative to it.

The Population Trajectory (Declining, Stable, Increasing, Unknown), is based on the best available knowledge about the current trajectory of each of the populations. Preliminary survey data shows that the density of Purple Wartyback in the Ausable River changed minimally over

[3] Museum records of this species exist from 1941, 1954 and 1975 but contain insufficient information to confirm.

three sampling periods from a watershed average of 0.18 (±0.12 SD) individuals/m^2 during initial quadrat surveys in 2006–2008 to 0.16 (±0.07) individuals/m^2 during follow-up surveys from 2011–2013, and to 0.18 (±0.14) individuals/m^2 during 2018–2022 surveys. The modeled population growth rate for the Ausable River over this time was 1.016 (CI: 0.985-1.049), which is not significantly different from one (i.e., stable; Appendix A). In the Sydenham River, density increased from a watershed average of 1.56 (±0.49) individuals/m^2 during initial quadrat surveys (1999–2003) to 2.69 (±0.91) individuals/m^2 during follow-up surveys (2012–2015); this increasing density was observed at eight of ten individual quadrat sites sampled (COSEWIC 2021). The modeled population growth rate for the Sydenham River was 1.047 (95% CI: 1.037-1.058) (van der Lee et al. in prep.[1]). Similarly in the Thames River, density increased from a watershed average of 0.10 (±0.05) individuals/m^2 during initial quadrat surveys (2004–2010) to 0.31 (±0.15) individuals/m^2 during the follow-up quadrat surveys (2015–2017); increasing density was observed at six of eight individual quadrat sites (COSEWIC 2021). The modeled population growth rate for the Thames River was 1.157 (95% CI: 1.10-1.221) (van der Lee et al. in prep.[1]). Analysis of population trends are depicted in Figure 4. These time series data are foundational in building population trajectories; however, the timespans between the initial and follow-up surveys are too short in the context of the generation time of this species (i.e., ~26 years) to draw strong conclusions about trajectory.

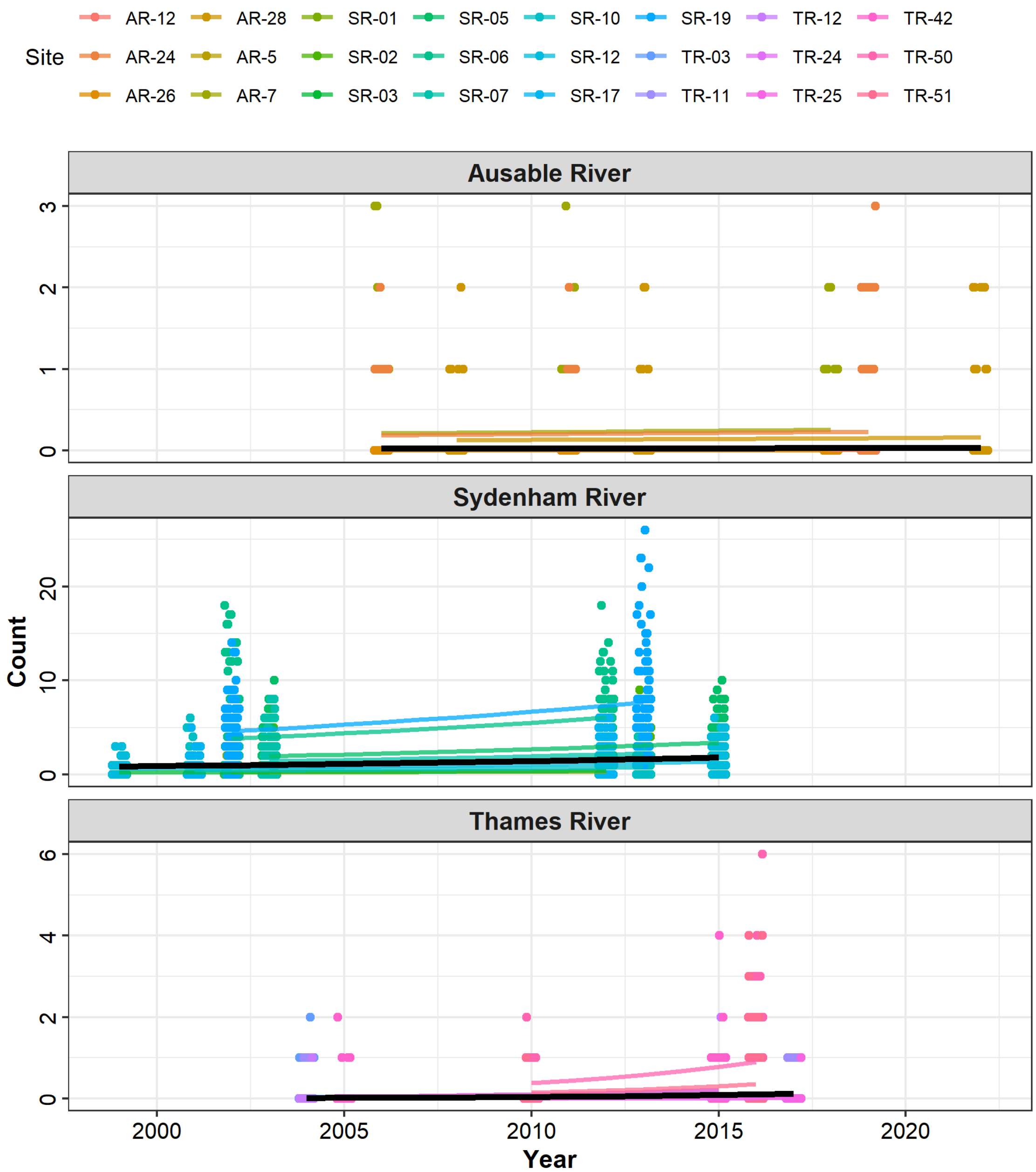

Figure 4. Population trends for the Ausable, Sydenham and Thames rivers resulting from an analysis of population density and trajectory at standardized quadrat sites in each river (van der Lee et al. in prep.[1]; Appendix A).

Table 2. Relative Abundance Index and Population Trajectory of each Purple Wartyback population in Canada. Certainty has been associated with each parameter based on 1 = quantitative analysis; 2 = CPUE or standardized sampling; 3 = expert opinion. Population growth rates (λ) for the Sydenham and Thames rivers are from van der Lee et al. (in prep.[1]), and were calculated for the Ausable River following the same methods (see Appendix A).

Population	Relative Abundance Index	Certainty	Population Trajectory*	Certainty
Ausable River	Low	1	Stable (λ = 1.016)	1
Sydenham River	High	1	Increasing (λ = 1.047)	1
Thames River	Medium	1	Increasing (λ = 1.157)	1

*population growth rates (λ) are based on data that spans 16, 16, and 13 years for the Ausable, Sydenham and Thames rivers, respectively, which is less than the most recent estimated generation time of the species of 26 years (van der Lee and Koops 2023); these values represent the current trend, but should not be taken as a definitive trajectory benchmark without additional years of data.

The Relative Abundance Index result is Low for the Ausable River due to the low density estimate and small occupied area, Medium for the Thames River due to medium density and large occupied area, and High for the Sydenham River due to the high density, and moderate to large occupied area.

The Relative Abundance Index and Population Trajectory rankings were then combined in the Population Status Matrix (Table 3) to determine the status for each population. Population Status was assigned as Poor, Fair, Good, or Unknown (Table 4) and the lowest level of certainty associated with either initial parameter was retained.

Table 3. The Population Status matrix combines the Relative Abundance Index and Population Trajectory rankings to establish the Population Status for each Purple Wartyback population in Canada.

		Population Trajectory			
		Increasing	Stable	Decreasing	Unknown
Relative Abundance Index	Low	Poor	Poor	Poor	Poor
	Medium	Fair	Fair	Poor	Poor
	High	Good	Good	Fair	Fair
	Unknown	Unknown	Unknown	Unknown	Unknown
	Extirpated	Extirpated	Extirpated	Extirpated	Extirpated

Table 4. Population Status of all Purple Wartyback populations in Canada, resulting from an analysis of both the Relative Abundance Index and Population Trajectory. Certainty assigned to each Population Status is reflective of the lowest level of certainty associated with either initial parameter (Relative Abundance Index, or Population Trajectory).

Population	Population Status	Certainty
Ausable River	Poor	1
Sydenham River	Good	1
Thames River	Fair	1

HABITAT AND RESIDENCE REQUIREMENTS

Element 4: *Describe the habitat properties that Purple Wartyback needs for successful completion of all life-history stages. Describe the function(s), feature(s), and attribute(s) of the habitat, and quantify by how much the biological function(s) that specific habitat feature(s) provided varies with the state or amount of habitat, including carrying capacity limits, if any.*

ADULT HABITAT

The Purple Wartyback inhabits medium to large rivers and occasionally deeper lake habitats. It is typically found in areas with moderate to swift currents, but is tolerant of slow flow and has been observed in dammed areas (Ostby 2005, Haggerty et al 1995). It is found at the substrate-water interface at a wide range of depths up to 6.0 m, and generally over coarser substrates of cobble, gravel, sand, and occasionally mud and cobble (Parmalee and Bogan 1998, COSEWIC 2021, DFO unpublished data). Purple Wartyback was associated with increasing water depth and lower shear stress in the Clinch River, Tennessee (Ostby 2005). A species distribution model of imperiled unionids in Michigan found that Purple Wartyback was positively associated with high stream flows, and negatively associated with high intensity urban land use in the catchment area, and amount of open river upstream of dams, the latter likely indicative of its tolerance to variable flow conditions (Daniel et al. 2018). From UMBO surveys and recent sampling events in Ontario (i.e., since 1997) where habitat data were collected, the mean water velocity was 0.376 m/s (range: 0.00–2.63 m/s), and mean water depth was 0.249 m (0.04–0.78 m) at sites with adult Purple Wartyback (> 55 mm). Substrate at these sites was composed of a mean of 32% (range: 0–90%) gravel, 26% (0–80%) sand, 24% (0–70%) cobble, 10% (0–80%) boulder, and 6% (0–60%) silt (LGLUD unpublished data).

Adult mussels are often found in multi-species mussel beds, assumed to be high-quality habitat patches. These beds are often found in river locations that have stable substrates under peak flows, but will remain wetted during low flows (Strayer 1999, Morales et al. 2006, Randklev et al. 1999). The location of mussel beds may also be related to hydrodynamics where juvenile mussels settle out into suitable habitat (French and Ackerman 2014, Lum 2020). These beds likely offer some protection in numbers from predation.

JUVENILE HABITAT

Little is known about habitat preferences of juvenile mussels in general, as they have relatively limited ability to select suitable habitat when dropping off their host fish (Schwalb and Ackerman 2011), or to relocate to more ideal habitat. Neves and Widlak (1987) evaluated microhabitat use of juvenile mussels in Big Moccasin Creek, Virginia (Purple Wartyback not present), and found significantly greater abundances of juvenile mussels in riffles and runs behind boulders than other habitat types. The majority of the juveniles collected were within 0–8 cm below the substrate surface. In Ontario, juvenile Purple Wartyback have been found in the same habitats as adults, buried in the sediment. During UMBO surveys from recent sampling events in Ontario where habitat data were collected, the mean water velocity was 0.373 m/s (range: 0–2.05 m/s) and the mean depth was 0.250 m (0.04–0.78 m) at sites where juvenile Purple Wartyback (≤55 mm) were collected. Substrate at these sites was composed of a mean of 33% (0–85%) gravel, 25% (0–75%) sand, 25% (0–70%) cobble, 10% (0–80%) boulder, and 5% (0–40%) silt.

GLOCHIDIAL HABITAT

Once glochidia are released by the female (loose on the mantle magazine or in a conglutinate), they must encounter a host fish, successfully attach to and encyst in gill tissue. Although research on Purple Wartyback host fishes has not been conducted in Canada, it is believed that the larger species of North American catfishes (Ictaluridae) serve as hosts (Hove et al. 1997, COSEWIC 2021). Bullheads (*Ameiurus* spp.) are ubiquitous through low-gradient, warmwater streams and wetlands (and warm, shallow bays of lakes) across much of southern Ontario, including the Ausable, Sydenham, and Thames rivers. They are benthic, and typically occupy heavily vegetated habitats with ample instream cover and soft substrates (Scott and Crossman 1998, Holm et al. 2009). Channel Catfish is known to occupy lakes and medium to large rivers through southern Ontario with coarser substrates (sand, gravel, cobble). Some individuals undertake long distance movements daily and/or seasonally (e.g., mean annual movement of 32.7–91.0 km (±28.0–93.8 km SD) in a Lake Winnipeg study), with periods of activity coinciding with the ice-off period (Scott and Crossman 1998, Enders et al. 2019). Additionally, Flathead Catfish has been identified as a potential host fish, and has been consistently detected in the lower Thames River since 2016, with several size classes represented indicating reproduction is likely occurring (Illes et al. 2019). Flathead Catfish prefers deep waters of medium to large rivers or lakes, with undercut banks and ample instream cover. Records of these species in southwestern Ontario from various DFO sampling efforts are depicted in Figure 5. The statuses of Black Bullhead, Yellow Bullhead and Channel Catfish are Secure or Apparently Secure in Ontario (NatureServe 2022), and all are considered pollution-tolerant, able to withstand a wide range of conditions (Scott and Crossman 1998), and as such, are unlikely to be limiting to Purple Wartyback completing its life cycle. A Michigan study found the distribution of Purple Wartyback was less limited by host availability than other unionids (Daniel et al. 2018).

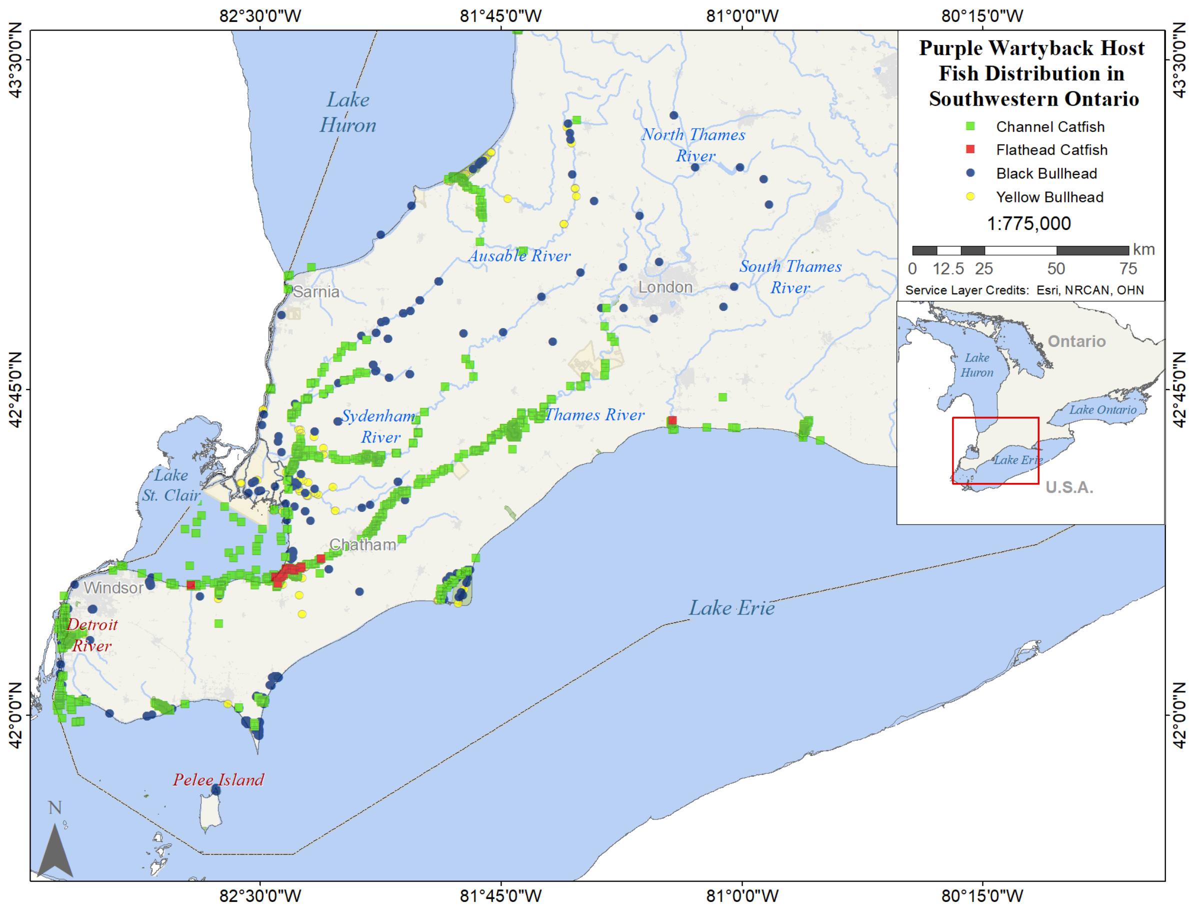

Figure 5. Records of bullheads, Channel Catfish, and Flathead Catfish in southwestern Ontario from various sampling efforts ([Biodiversity Science Database]). Note these records may not represent the full distribution of the species in southwestern Ontario.

FUNCTIONS, FEATURES, ATTRIBUTES

A description of the essential functions, features, and attributes associated with the habitat of Purple Wartyback in Canada are described to guide identification of critical habitat for this species (Table 5). The habitat required for each life stage has been assigned a life-history function that corresponds to a biological requirement of Purple Wartyback. In addition to the life-history function, a habitat feature has been assigned to each life stage. A feature is considered to be the structural component of the habitat necessary for the species to complete its life cycle. Habitat attributes have also been provided, these are measurable components describing how the habitat features support the life history function for each life stage. Optimal habitat attributes from the literature have been combined with attributes from recent records to show the range of habitat values that Purple Wartyback may be found in (note that the species may be currently occupying areas where habitat is not optimal).

Table 5. Summary of the essential functions, features, and attributes for each life stage of Purple Wartyback in Canada. Habitat attributes from the published literature and those recorded during recent sampling events can be used to support delineations of critical habitat.

Life Stage	Function	Feature	Attribute		Critical Habitat
			Scientific Literature	Recent Knowledge	-
Spawning and fertilization (spring through early summer)	Reproduction	Reaches of small to large rivers	Substrate of cobble, gravel, small boulders; water depth 0.4–1.2 m; water temperature ≥ 9°C (Jirka and Neves 1992)	-	Reaches of medium to large rivers with moderate to swift current and sand, gravel and cobble substrates.
Encysted glochidial stage (late summer through fall)	Feeding Cover Nursery	Same as above with host fishes present (presumed host fishes include: Black and Yellow bullheads, Channel Catfish, possibly Flathead Catfish)	Black and Yellow bullheads: low gradient streams, shallow, warmwater bays of lakes and wetlands; Channel and Flathead catfishes: medium to large rivers or deeper areas of lakes with ample vegetation and in stream cover (e.g., coarse woody debris) (Scott and Crossman 1998, Holm et al. 2009)	Water chemistry: mean conductivity = 558.6 µs/cm (range: 5.49–863 µs/cm); mean dissolved oxygen = 9.07 mg/L (5.68–20 mg/L); mean pH = 8.32 (7.19–9.12); physical parameters: mean depth = 2.07 m (0.26–9.60 m); mean water velocity = 0.02 m/s (0–0.26 m/s); mean stream width = 93 m (11–233 m); mean coarse woody debris cover = 13% (0–60%); Mean substrate composition: 39% (0–95%) clay, 36% (0–80%) silt, 13% (0–80%) organic, 9% (0–100%) sand. (Biodiversity Science Database from Ausable, Sydenham and Thames rivers where host fishes occur)	Same as above. Presence of sufficient host fishes.
Juvenile (age 0 to approximately age 7 or 55 mm)	Feeding Cover Nursery	Reaches of small to large rivers with a combination of soft and hard substrates suitable for burrowing	-	Mean water velocity of 0.373 m/sec (range: 0.00–2.05 m/s); Mean depth of 0.250 m (0.04–0.78 m); Mean substrate composition of 33% (0–85%) gravel, 25% (0–75%) sand, 25% (0–70%) cobble, 10% (0–80%) boulder, and 5% (0–40%) silt. (LGLUD unpublished data)	Same as above

Life Stage	Function	Feature	Attribute		Critical Habitat
			Scientific Literature	Recent Knowledge	-
Adult (> age 7 or 55 mm)	Feeding Cover	Reaches of small to large rivers	Variable substrate of sand, gravel, cobble, small boulders, occasionally silt; flow nearly absent to swift; (Jirka and Neves 1992, Haggerty et al. 1995); depths from 0.6–6.0 m (Parmalee and Bogan 1998, COSEWIC 2021)	mean water velocity 0.376 m/s (range: 0.00–2.63 m/s); mean water depth 0.249 m (0.04–0.78 m); mean substrate composition: 32% (0–90%) gravel, 26% (0–80%) sand, 24% (0–70%) cobble, 10% (0–80%) boulder, and 6% (0–60%) silt. (LGLUD unpublished data)	Same as above

Element 5: *Provide information on the spatial extent of the areas in Purple Wartyback's distribution that are likely to have these habitat properties.*

As the understanding of Purple Wartyback habitat is fairly general, most of its distribution in Ontario is likely to contain patches or reaches of suitable habitat, but the spatial extent has not been explicitly mapped. Areas likely to contain suitable substrate and flow conditions are considered the stretch between Nairn and Arkona in the Ausable River amounting to approximately 62.2 km; a nearly continuous stretch in the East Sydenham River of 85.9 km from Napier to downstream of Dresden; and 9.6 km from Plover Mills to upstream of Fanshawe Lake on the North Thames River, 23.6 km from Dorchester to the Forks (Hunt Dam) in London on the South Thames River, and 136.0 km from Delaware to Kent Bridge (possibly an additional 46.9 km to the mouth) on the mainstem of the Thames River. It is unlikely that the entirety of these stretches is suitable, and likely that other suitable habitat exists beyond these known river segments.

Element 6: *Quantify the presence and extent of spatial configuration constraints, if any, such as connectivity, barriers to access, etc.*

There are several physical barriers that could prevent Purple Wartyback and its host fishes from dispersing or accessing new habitats. There are two major dams, Exeter Dam and Morrison Dam, on the Ausable River located approximately 25 km upstream of Nairn, the upstream end of the known Purple Wartyback distribution (COSEWIC 2021, K. Jean pers. comm.). There are two dams located upstream of Napier on the Sydenham River, at Head Street in Strathroy (53 km upstream of Alvinston) and Coldstream Dam in Greystead (72 km upstream of Alvinston), and an additional 11 smaller dams and barriers through the distribution of Purple Wartyback. The Sydenham River also has one of the largest flood control structures in Ontario, the McKeough Floodway, located 12 km north of Wallaceburg, Ontario. This structure controls approximately 37% of the East and North Sydenham River drainages (SCRCA 2018, SCRCA 2022). The gates remain open during normal flow conditions, but close during high flow events and water overflows into a floodway channel. Fishes and mussels could be swept into the diversion channel during these events. There are three major and 10 small to medium dams in the upper Thames River watershed that could impact dispersal, four of these occur within the distribution of Purple Wartyback, and another five occur > 40 km upstream of the known distribution. The Fanshawe Dam is completely impassable for fishes and effectively isolates the North Thames River subpopulation of Purple Wartyback from the rest of the Thames River. Hunt Dam is located 2 km upstream of the Forks in London and may limit passage of fishes and mussels between the South Thames River and other branches. There are an additional 225 barriers within the watershed (UTRCA 2022). In all three watersheds, the extent to which these smaller dams and barriers prevent movement of aquatic animals is unknown.

There are no major physical barriers preventing Purple Wartyback or its host fishes from moving between populations (including extirpated locations). Habitat through the Huron-Erie Corridor (i.e., lower Lake Huron, St. Clair River, Lake St. Clair, Detroit River into western Lake Erie) is likely unsuitable and distances too great for bullhead hosts to travel, but may not limit Channel Catfish. Dreissenid mussels, however, may create a biological barrier to dispersal, as habitat in the Great Lakes and connecting channels is still mostly inhospitable for unionid mussels due to the presence of dreissenids. Purple Wartyback appears to be tolerant of a range of environmental conditions, but its very limited distribution in southwestern Ontario suggests there are likely habitat or other environmental variables that are important to the species that are yet unknown.

Element 7: *Evaluate to what extent the concept of residence applies to the species, and if so, describe the species' residence.*

Residence is defined in SARA as a “dwelling-place, such as a den, nest or other similar area or place, that is occupied or habitually occupied by one or more individuals during all or part of their life cycles, including breeding, rearing, staging, wintering, feeding or hibernating”. Residence is interpreted by DFO as being constructed by the organism (DFO 2010). In the context of the above narrative description of habitat requirements during glochidial, juvenile and adult life stages, Purple Wartyback does not construct a residence during its life cycle.

THREATS AND LIMITING FACTORS TO THE SURVIVAL AND RECOVERY OF PURPLE WARTYBACK

Element 8: *Assess and prioritize the threats to the survival and recovery of the Purple Wartyback.*

Freshwater mussels are among the most imperiled taxa in the world. Approximately 72% of species in North America are of conservation concern, due to widespread habitat alteration, pollution and AIS (particularly dreissenid mussels) (Bogan 1993, Williams et al. 1993, Metcalfe-Smith et al. 1998, Ricciardi and Rasmussen 1999). A number of threats may limit the survival and recovery of Purple Wartyback in Canada. Pollution from agricultural and urban sources, impacts of climate change (e.g., droughts), biotic interactions from AIS, and dredging are considered the greatest threats to this species (COSEWIC 2021). Although Purple Wartyback is dependent on host fishes, likely larger-bodied ictalurids, for completing its life cycle, the threat assessment does not consider threats to those species. Catfishes are known to be pollution-tolerant, warmwater species that spawn in variable habitats during late spring (Scott and Crossman 1998) and are likely to be impacted to a lesser extent than Purple Wartyback from the effects of pollution or climate change. These fishes may compete with AIS like Round Goby (*Neogobius melanostomus*) for benthic prey, but their omnivorous/opportunistic feeding strategy likely reduces competitive interactions. Barriers to fish passage on the Ausable, Sydenham, and Thames rivers are not likely a threat to catfish hosts completing their own life cycle, but could represent an impediment to the dispersal of Purple Wartyback glochidia. Threats are categorized following COSEWIC (2021), based on Salafsky et al. (2008).

POLLUTION

As sedentary filter-feeders, freshwater mussels are generally vulnerable to the effects of pollution both in the water column and in the sediment. Glochidia and juvenile mussels are most sensitive to contaminant effects, while adults are better able to withstand acute exposures with behavioural avoidance (valve closure/burrowing) with fewer metabolic consequences (Shick et al. 1988, Byrne et al. 1990, Cope et al. 2008). There is some evidence that mucous-encased glochidia conglutinates may be afforded protection against some contaminants (Gillis et al. 2008, Gillis 2011). Early life stages are the most sensitive to pollution (Gillis et al. 2008, Gillis 2011). However, pollutants that impact adult Purple Wartyback are the most likely to result in population-level declines (van der Lee and Koops 2023). To our knowledge, Purple Wartyback has not been used during toxicity testing and specific contaminant effects are unknown for this species, but it likely falls within known sensitivity ranges published for other freshwater mussel species (Raimondo et al. 2016).

Agricultural and Forestry Effluents

The three watersheds with Purple Wartyback populations in Ontario all have intensive agricultural land use, ranging from ~70–80%, with a high proportion of watercourses being channelized, tiled or buried drains (ABCA 2018a,b, SCRCA 2018, UTRCA 2017a,b,c,d). Approximately 4700 km of watercourses in the Sydenham River watershed are classified as drains (60% of watercourses), and 4800 km in the lower Thames River. These watersheds have nutrient levels that often exceed provincial guidelines (Appendix D), and riparian buffers that would filter agricultural run-off are often poorly established (Poole and Downing 2004).

Siltation/sedimentation of water courses is a widespread threat facing aquatic life in southwestern Ontario, and can arise from many agricultural sources including livestock access to rivers, poor soil retention practices, erosion/bank stability issues, and is made worse by a lack of riparian buffers. Sediments may be suspended leading to high turbidity, or settle out and deposit on coarser substrates and live animals. Suspended sediments can clog incurrent siphons and gills, interrupting feeding, respiration, growth, and reproduction (Tuttle-Raycraft et al. 2017, Goldsmith et al. 2021, Luck and Ackerman 2022). As mussels filter water, they remove non-food items for expulsion, and in highly turbid areas, sorting sediments out may become too energetically costly compared to the food coming in (Madon et al. 1998, Tokumen et al. 2016, Tuttle-Raycraft and Ackerman 2019). Sediments can clog the gills, resulting in reduced respiration rates and oxygen uptake (Madon et al. 1998, Aldridge et al. 1987). High loads of total suspended solids (TSS) can reduce fertilization success in females; increased production of pseudofeces can result in sperm being expelled before being captured. In one study, a short-term brooder that uses all four sets of gills for brooding glochidia experienced greater respiratory stress compared to a long-term brooder that uses only two sets of gills (Gascho Landis and Stoeckel 2016); however, Purple Wartyback appears to use only the outer two demibranchs (Campbell et al. 2005). Osterling et al. (2010) found reduced recruitment of juveniles, and reduced adult growth in stream systems with 3-4 times the turbidity. High suspended sediment loads may decrease mussel-host fish interactions, particularly when lures, mantle displays, and prey-mimicking conglutinates are used to attract host fishes (Goldsmith et al. 2021), although Purple Wartyback may be less affected than other species when using non-specific conglutinate masses. Heavy sediment loads may also make host fish less susceptible to infestation as fish gill tissue may become damaged from abrasive sediments, or mucous secreted to protect gill tissue from abrasion may reduce attachment and metamorphosis rates (Goldsmith et al. 2021 from Beussink 2007). For buried mussels, deposited silt and other fines can clog interstitial space, reducing feeding (for juveniles) and respiration success, and may impact burrowing activity or lead to death (Brim Box and Mossa 1999).

Nutrient loading is another consequence of agricultural land use that can negatively affect mussels and fish hosts. Nutrients can come from a number of agricultural sources, including fertilizers and manure, and may become resuspended during drain maintenance activities or when cattle are accessing streams. Nutrients increase primary productivity, particularly algal growth, which can lead to reduced dissolved oxygen both daily and seasonally (i.e., during periods of decomposition). This can impact respiration and potentially lead to mortality at extreme levels of anoxia (Sparks and Strayer 1998). Fertilizers and other nitrogenous compounds can result in increased ammonia levels in the aquatic environment, and freshwater mussels are among the most sensitive taxa to ammonia, particularly at early life stages (Augspurger et al. 2003, Wang et al. 2007). Potassium, often found in fertilizers, is also toxic to early life stage mussels (Gillis et al. 2021). Concentrations of ammonia and potassium in Ontario rivers occasionally exceed the EC_{10} (concentration at which 10% of individuals exhibit an effect) for glochidia of Rainbow (*Cambarunio iris*), another mussel of conservation concern (Salerno et al. 2020).

Lastly, pesticides applied to farm fields or occasionally in or near water for aquatic invasive species control (e.g., glyphosate for *Phragmites australis australis*) may also be toxic to freshwater mussels, depending on exposure concentration (Keller and Ruessler 1997, Bringolf et al. 2007), and genotoxic effects have also been reported (Conners and Black 2004). However, probabilistic risk assessments based on measured levels of widely used pesticides (neonicotinoids, fungicides, carbamates, organophosphates and butenolides) in Ontario waters and each of their toxicities to freshwater mussels (Fat Mucket [*Lampsilis siliquoidea*], Wavy-rayed Lampmussel [*Lampsilis Fasciola*], and Rainbow) revealed mussels were insensitive to the 13 pesticides tested and none were considered to pose a risk at current environmental levels (Prosser et al. 2016, Salerno et al. 2018). Bayluscide (niclosamide ethanolamine salt) is a chemical lampricide applied strategically throughout the Great Lakes basin for control of Sea Lamprey (*Petromyzon marinus*) and could negatively affect sensitive native species[4]. An evaluation of the relative risks of granular Bayluscide applications to at-risk fishes and mussels was recently conducted by Andrews et al. (2021), and although Purple Wartyback was not included, the risks to closely related and overlapping species suggest the risk to Purple Wartyback is likely low at this time. Closely-related Mapleleaf had a relatively low toxicity score (only 3% mortality was observed in individuals exposed to environmentally relevant concentrations (Newton et al. 2017)), and Kidneyshell (*Ptychobranchus fasciolaris*), which has a very similar current distribution, showed very little spatial overlap with applications of granular Bayluscide from 2011–2017 (Andrews et al. 2021).

Domestic and Urban Wastewater

The majority of land use surrounding the Ausable, Sydenham and Thames rivers is agricultural, but these systems are not immune from the effects of urbanization, and urban development is expected to continue increasing in southwestern Ontario. Urban wastewater and runoff can result in numerous point and non-point sources of pollutants that are of concern to freshwater mussels.

Road salts applied for winter de-icing are a major concern for freshwater mussels, as chloride is among the most toxic substances to unionids particularly at the glochidial stage (Gillis 2011, Pandolfo et al. 2012b). Todd and Kaltenecker (2012) reported maximum chloride values measured in Ontario rivers (including the Ausable and Thames) exceeded the EC20 tolerances of glochidia reported during lab tests (Gillis 2011). Using modeled chloride exposure distributions and species sensitivity distributions, Prosser et al. (2017) determined there was an approximately 97% probability that chronic chloride levels experienced in the Sydenham and Thames rivers exceed the level at which 95% of mussel species would be negatively affected. This is likely of greatest concern in the Thames River where road density and urban land use are highest (Todd and Kaltenecker 2012, Sorichetti et al. 2022). Gillis et al. (2022) reported low viability of glochidia exposed to winter road runoff from samples collected in the Thames River watershed during lab trials related to high chloride concentrations (and possibly amplified when potassium was also present). Other contaminants associated with roadways (e.g., polycyclic aromatic hydrocarbons [PAHs] and heavy metals) are likely to negatively affect feeding, behaviour, reproduction, and growth, but can also have toxic and mutagenic effects on freshwater mussels (Keller and Zam 1991, Marvin et al. 1994, Naimo 1995, Archambault et al. 2018).

There are numerous wastewater or sewage treatment plants found in the Ausable (n=14), Sydenham (n=18) and Thames (n=30) watersheds that could negatively affect Purple Wartyback (ABCA 2018a,b, SCRCA 2018, UTRCA 2017a,b,c,d). Most wastewater is treated prior to being released into rivers, but not all contaminants are removed. Gillis et al. (2017) reported a complete absence of mussels for an approximately 7 km stretch downstream of a large (>200,000 households serviced) wastewater treatment plant on the Grand River, Ontario,

[4] Note that lampricide is applied to control AIS, and although not used for agriculture or forestry purposes, its function as a pesticide with impacts to non-target organisms is best captured in this category.

in contrast to a healthy mussel community immediately upstream, likely related to high nitrite and ammonia and low dissolved oxygen. In addition to ammonia, municipal wastewater effluent often contains other potentially toxic compounds like pharmaceuticals and personal care products. Estrogenic compounds can lead to feminization and other neuroendocrine disruptions in mussels and fishes, resulting in reproductive consequences (Gagné et al. 2004, Gagné et al. 2011, Tetreault et al. 2011). Although dozens of pharmaceuticals and personal care products have been detected in the tissues of wild mussels (de Solla et al. 2016), toxicity assessments found in Ontario municipal wastewater effluents revealed that none were toxic to mussels at the levels found in the environment; however, some behavioural effects were observed (Gilroy et al. 2014, 2017). Additionally, contaminants found in urban runoff (e.g., heavy metals) may interact with those found in wastewater effluent leading to reduced body condition and longevity in mussels found downstream of these inputs (Gillis 2012, Gillis et al. 2014). Microplastics from urban and industrial sources are also appearing in surface waters and sediments around the Great Lakes basin (Driedger et al. 2015, Dean et al. 2018) and have been documented in Flutedshell (*Lasmigona costata*) in the Grand River (Wardlaw and Prosser 2020). Weir et al. (in prep.[5]) found that different mussel tissues accumulated microplastics to different degrees, and this would dictate which (if any) biological functions are impaired. Faulty or leaching septic systems have also been identified as an issue in the Ausable and Sydenham watersheds (SCRCA 2018, ABCA, pers. comm.). These can contribute nutrients leading to increased algal blooms and decreased dissolved oxygen.

CLIMATE CHANGE AND SEVERE WEATHER

Freshwater mussels are generally considered vulnerable to impacts of climate change, owing to their reliance on host fishes to complete their life cycle and a limited ability to disperse to new habitats if conditions become unfavourable (Brinker et al. 2018). However, the degree to which climate change will affect Purple Wartyback in Canada is unknown, and although climate change impacts are measurable in Ontario, no impacts to Purple Wartyback are measurable at this time (COSEWIC 2021). Considerable changes in temperature and precipitation are expected across Ontario by 2100, with mean annual temperatures expected to increase, summers to see a decrease in total precipitation and winters an increase compared to previous decades (McDermid et al. 2015). Climate change may have indirect impacts on mussels and mussel habitat (e.g., including increases in nutrient and turbidity loads, altered flow regimes and changes to water velocity, increased disease prevalence, changes in distribution of competitors and/or predators) (Lemmen and Warren 2004, COSEWIC 2021).

Droughts

The most significant impact of climate change for Purple Wartyback is expected to be a reduction of habitat quantity and quality due to increasing frequency and severity of droughts. Droughts will result in a loss of habitat space, increased risk of desiccation, increased predation risk from terrestrial and avian predators, and density-dependent effects like reduced food supply through competition, increased risk of disease transfer due to crowding, and reduced dissolved oxygen through consumption. Low flows during droughts can also lead to increased temperatures, decreased dissolved oxygen, and higher turbidity. Following a severe drought event in Georgia, mussel abundance declined by at least 50% compared to pre-drought surveys in six of the most impacted reaches, but reaches with coarse woody debris where shallow

[5] Weir, E., Robson, E., Prosser, R., Gillis, P., Bennett, J, Salerno, J., and Kidd, K. In prep. Using wild-caught freshwater mussels as bio-indicators for microplastic accumulation downstream of municipal wastewater treatment plants in the Grand River watershed. In preparation.

depressions formed appeared to offer some refuge to mussels (Golladay et al. 2004). Similarly, in an Oklahoma river, drought-sensitive mussels (including Pimpleback [*Cyclonaias pustulosa*]) declined by 67% (compared to no change in abundance for drought-tolerant species) during a study period where the number of drought days increased by 37% and coincided with increased air temperatures (Lopez et al. 2022). Purple Wartyback is considered tolerant of slow flow (Ostby 2005) and is likely more resilient during drought conditions than lotic-specialist species.

In addition to droughts, extreme flood events could potentially flush mussels to less ideal habitats, and scour stream beds, changing substrate composition in suitable patches. If floods occur during spawning season, the uptake of sperm by females could be limited, resulting in reduced fertilization success, or availability of conglutinates could be impacted, preventing larval development. All three rivers inhabited by Purple Wartyback in Canada are considered flashy due to the high degree of semi-impervious surfaces in the watersheds (ABCA 2018a,b, SCRCA 2018, UTRCA 2017a,b,c,d).

Temperature Extremes

Heat waves are also likely to increase in frequency and intensity with climate change. In lab trials meant to simulate conditions of a heat wave, freshwater mussels experienced a physiological response (e.g., changes to clearance rates) and accrued a cost associated with upregulating heat shock proteins to withstand acute exposures to temperature increases (Payton et al. 2016, Ferreira-Rodriguez et al. 2018). Mass mortality events are an extreme, though not improbable outcome of exposures to heat waves, but long term consequences of repeat exposures to heat extremes at a population level are still poorly understood for freshwater species. The lethal thermal tolerance and thermal optimum of Purple Wartyback are not known. Pandolfo et al. (2010) reported a mean upper lethal limit of 33.1°C (range 21.4–42.7°C) for eight species of freshwater mussels (glochidia and juvenile), while Martin (2016) reported higher tolerances, ranging 33.2–40.8°C for three species (noting differences in acclimation temperature and age); however, host fish thermal tolerance may be more limiting in some cases (Pandolfo et al. 2012a). Upper lethal thermal tolerances of ictalurid catfishes have been reported as 33.5–37.5°C, depending on species and acclimation temperature (Scott and Crossman 1998). Although the putative catfish hosts of Purple Wartyback are tolerant of a range of conditions, this glochidial-host relationship can be precarious under ideal conditions, and climate change could result in mismatches in timing of mussel spawning and host site occupancy, host feeding behaviours, host health and susceptibility to infestation.

INVASIVE AND OTHER PROBLEMATIC SPECIES AND GENES

Invasive Non-native/Alien Species/Diseases

The invasion of dreissenid mussels (Zebra Mussel and Quagga Mussel) in the Great Lakes basin resulted in the near eradication of native unionid mussels in the lakes, connecting channels, and lower reaches of tributaries by the mid 1990’s (Gillis and Mackie 1994, Schloesser and Nalepa 1994, Nalepa et al. 1996, Ricciardi et al. 1996, Schloesser et al. 2006). Dreissenids attach to native mussels via byssal threads and can accumulate on their shells in extremely large numbers. This can smother the siphon (reducing feeding and respiration), prevent or inhibit valve movements, interfere with burrowing activities, and impair shell formation (Gillis and Mackie 1994, Nalepa et al. 1996, Schloesser et al. 2006). Dreissenid mussels also appear to outcompete native unionids for food resources. Nalepa et al. (1996) found that the filtering capacity of the Detroit River Zebra Mussel population was approximately 12 times greater than that of the native unionid population several years earlier, despite a lower mean biomass of Zebra Mussel. Dreissenid mussels are the likely cause of Purple Wartyback extirpation in the Detroit River and western Lake Erie; however, there has been recent evidence to suggest the impacts of dreissenids on native unionids are lessening (Crail et al. 2011). Dreissenid mussels are lentic species, typically found in low abundances in riverine habitats as they have poor attachment abilities under flowing conditions (Horvath et al. 1996, Stoeckel et al. 1997). Purple Wartyback found in the Ausable, Sydenham and Thames rivers are at relatively low risk of impacts from these invasive mussels, although Zebra Mussel has been detected in the lower reaches of the Sydenham River (below the distribution of Purple Wartyback), in Fanshawe Lake on the North Thames River, and from the Forks to Thamesville on the lower Thames River, including attached to live unionids (Morris and Edwards 2007).

Round Goby is a small-bodied benthic fish native to the Ponto-Caspian Sea (like dreissenid mussels) that has expanded its range through the Great Lakes basin. It now occupies reaches of the Ausable River where Purple Wartyback is found, and the full extent of the Purple Wartyback distribution in the Sydenham and Thames rivers (Poos et al. 2010, K. Jean pers. comm.). Round Goby may prey on juvenile mussels (Clark et al. 2022), but negative effects on host fishes (i.e., through competition and/or predation) is likely the larger impact to unionids (Poos et al. 2010). Round Goby may compete with or prey on small-bodied ictalurids (i.e., madtoms *Noturus*; French and Jude 2001). Additionally, Round Goby may occasionally become infested with glochidia of native unionids, but it appears to offer poor metamorphosis success (Tremblay et al. 2016). The extent to which Round Goby predates on Purple Wartyback, impacts host interactions, or functions as a sink for glochidia is unknown.

Ballast water regulations and monitoring have improved considerably in Canada in recent years (Bailey et al. 2011), largely following the impacts of dreissenid mussels and Round Goby, and it is hoped that these will curtail the arrival of similar species in the future. Other pathways (e.g., aquarium/ ornamental trade, spread from connecting waterbodies) could still result in the arrival and spread of new AIS of concern. Notably, Black Carp (*Mylopharyngodon piceus*) is a molluscivore that has established in the Mississippi River that could pose significant threats to native unionids should it arrive in the Great Lakes (Nico et al. 2005); however, it is unlikely to arrive within the 10-year timeframe considered here.

NATURAL SYSTEMS MODIFICATIONS

Other Ecosystem Modifications

Dredging for agricultural drain maintenance occurs in tributaries upstream of Purple Wartyback habitat in all three watersheds it occupies, and is another contributor of sediments and nutrients that are transported downstream and may accumulate in the main channel of the rivers. Dredging associated with drain maintenance can also cause direct mortalities of mussels through physical damage to individuals or by being buried in dredgeate piles (Killeen et al. 1998, Aldridge et al. 2000); however, dredging is not thought to occur in main channel reaches occupied by Purple Wartyback in Ontario. Impacts of sedimentation and nutrient resuspension are discussed elsewhere, but given these dredging activities are not occurring directly in Purple Wartyback habitat, this threat is not assessed.

TRANSPORTATION AND SERVICE CORRIDORS

Roads and Railways

Bridge and culvert construction or maintenance projects have the potential for direct and indirect local effects, especially if mussel relocations are not conducted with in-stream works. Impacts may include: mortalities, increased turbidity, altered substrate and flow regimes, streambank erosion, altered nutrient and food resources, and loss of connectivity for fish hosts (Wheeler et al. 2005, Cocchiglia et al. 2012). These activities have been reported in the Sydenham River (n=1) and the Thames River (n=9) within the range of Purple Wartyback in the last 10 years. Although local effects could be severe, population-level impacts are unlikely, and thus, the overall impact of this threat is thought to be negligible. Should these activities increase in frequency, occur without relocations, or project planning does not account for high density patches of mussels, this threat should be reconsidered.

HUMAN INTRUSTIONS AND DISTURBANCES

Recreational Activities

Recreational vehicle (e.g., ATV) use within streams can cause impacts to the surrounding riparian areas through soil compaction, damaged vegetation, and transport of invasive species; and to water quality (through increased pollution and turbidity), stream bed composition, and cause mortality of aquatic animals (Kidd et al. 2014, Cooke and Xia 2020, Goodwin et al. 2021). ATV use in aquatic habitats has caused turtle nest mortality, especially when nests were located in shallower reaches and when ATVs were driven more slowly over them or turned on them (Godwin et al. 2021). ATV use within streams is known to occur in all three river systems occupied by Purple Wartyback (K. Jean, ABCA, pers. comm., C. Paterson, SCRCA, pers. comm., V. McKay, LTVCA, pers. comm.). Given the localized area of impact, these activities are unlikely to have population-level effects but may still result in harm to individuals even though Purple Wartyback is a thick-shelled, robust mussel. This threat is thought to have a negligible impact on Purple Wartyback persistence at this time, but should be reconsidered if activities increase in frequency or intensity.

MULTIPLE THREAT EFFECTS

The areas inhabited by Purple Wartyback are likely experiencing multiple threats concurrently, which may interact in complex and context-dependent ways. This could result in additive effects, or stressors could be amplified or dampened in combination compared to each threat on its own. As such, the magnitude and direction of impacts are difficult to predict, but research on multiple threat effects is growing. Luck and Ackerman (2022) evaluated the interactive effects of water temperature, water velocity, and total dissolved solids on three measures of mussel ecophysiology and found that, in several cases, combining stressors resulted in multiplicative effects. A worst case scenario was identified of high summer temperatures combined with heavy turbidity and either above- or below-average velocity, which are likely conditions under most climate change scenarios where droughts or intense rain events are expected to increase (Luck and Ackerman 2022). Beermann et al. (2021) also generally found negative synergistic effects on benthic invertebrate communities when suspended sediments increased and flow velocity decreased; sensitive taxa were further impacted when salinity increased as well. Contaminants ammonia, chloride, copper, and potassium are known to be among the most toxic to freshwater mussels in isolation, but likely co-occur (along with other stressors) in the natural environment. Salerno et al. (2020) investigated mixture toxicity of pairings of these contaminants on early life stage mussels, and found that they typically resulted in synergistic effects (depending on exposure level) in combination compared to individual exposures. Further investigation of multiple stressors on mussel vital rates is warranted.

THREAT ASSESSMENT

Threats were assessed following guidelines in DFO (2014). Each threat was ranked in terms of the threat Likelihood of Occurrence (LO), threat Level of Impact (LI) and Causal Certainty (CC). The Likelihood of Occurrence was assigned as Known, Likely, Unlikely, Remote or Unknown, and refers to the probability of a specific threat occurring for a given population over 10 years or 3 generations, whichever is shorter. Given the long generation time for Purple Wartyback (26 years) this threat assessment was evaluated over a 10 year time frame. The Level of Impact was assigned as Extreme, High, Medium, Low, or Unknown and refers to the magnitude of the impact caused by a given threat, and the level to which it affects the survival or recovery of the population. The level of certainty associated with each threat was assessed and classified as: 1 = very high, 2 = high, 3 = medium, 4 = low, 5 = very low. The Population-Level Threat Occurrence (PTO), Threat Frequency (PTF) and Threat Extent (PTE) were also evaluated and assigned a status based on the definitions outlined in Table 6 (rankings in Table 7). The Likelihood of Occurrence and Level of Impact for each population were subsequently combined in the population-level Threat Risk Matrix (Table 8; rankings in Table 9). The species-level Threat Assessment in Table 10 is a roll-up of the population-level threats identified in Table 9.

Table 6. Definition and terms used to describe likelihood of occurrence (LO), level of impact (LI), causal certainty (CC), population-level threat occurrence (PTO), threat frequency (PTF) and threat extent (PTE) reproduced from DFO (2014).

Term	Definition
Likelihood of Occurrence (LO)	
Known or very likely to occur (K)	This threat has been recorded to occur 91-100%
Likely to occur (L)	There is a 51-90% chance that this threat is or will be occurring
Unlikely (UL)	There is 11-50% chance that this threat is or will be occurring
Remote (R)	There is 1-10% or less chance that this threat is or will be occurring
Unknown (U)	There are no data or prior knowledge of this threat occurring or known to occur in the future
Level of Impact (LI)	
Extreme (E)	Severe population decline (e.g., 71-100%) with the potential for extirpation
High (H)	Substantial loss of population (31-70%) or threat *would jeopardize* the survival or recovery of the population
Medium (M)	Moderate loss of population (11-30%) or threat is *likely to jeopardize* the survival or recovery of the population
Low (L)	Little change in population (1-10%) or threat is *unlikely to jeopardize* the survival or recovery of the population
Unknown (U)	No prior knowledge, literature or data to guide the assessment of threat severity on population
Causal Certainty (CC)	
Very high (1)	Very strong evidence that threat is occurring and the magnitude of the impact to the population can be quantified
High (2)	Substantial evidence of a causal link between threat and population decline or jeopardy to survival or recovery
Medium (3)	There is some evidence linking the threat to population decline or jeopardy to survival or recovery
Low (4)	There is a theoretical link with limited evidence that threat is leading to a population decline or jeopardy to survival or recovery
Very low (5)	There is a plausible link with no evidence that the threat is leading to a population decline or jeopardy to survival or recovery
Population-Level Threat Occurrence (PTO)	
Historical (H)	A threat that is known to have occurred in the past and negatively impacted the population.
Current (C)	A threat that is ongoing, and is currently negatively impacting the population.

Term	Definition
Anticipatory (A)	A threat that is anticipated to occur in the future, and will negatively impact the population.
Population-Level Threat Frequency (PTF)	
Single (S)	The threat occurs once.
Recurrent (R)	The threat occurs periodically, or repeatedly.
Continuous (C)	The threat occurs without interruption.
Population- Level Threat Extent (PTE)	
Extensive (E)	71-100% of the population is affected by the threat.
Broad (B)	31-70% of the population is affected by the threat.
Narrow (N)	11-30% of the population is affected by the threat.
Restricted (R)	1-10% of the population is affected by the threat.

Table 7. Threat Likelihood of Occurrence (LO), Level of Impact (LI), Causal Certainty (CC), Population-level Threat Occurrence (PTO), Population-level Threat Frequency (PTF), and Population-level Threat Extent of each Purple Wartyback population in Canada. Definitions and terms used to describe the threat ratings are found in Table 6. Additional rationale for scores presented in (d).

a) Ausable River

IUCN Threat Category	Sub-category	Details	Likelihood of Occurrence	Level of Impact	Causal Certainty	Threat Occurrence	Threat Frequency	Threat Extent
Pollution	Agricultural and Forestry Effluents	Sedimentation (field runoff, upstream drain maintenance)	K	L	5	H/C/A	C	B
		Nutrient Loading (+ ammonia)	K	L	5	H/C/A	C	B
		Pesticides (+ granular Bayluscide)	K	L	5	H/C/A	C	B
	Domestic and Urban Wastewater (incl. urban runoff)	Nutrient Loading (+ ammonia)	K	L	5	H/C/A	C	B
		Pharmaceuticals and estrogenic compounds	K	L	5	H/C/A	C	B
		Chloride	K	L	5	H/C/A	R	B
	-	Heavy Metals	K	L	5	H/C/A	C	B
Climate Change and Severe Weather	-	Frequent and severe droughts and heat waves	K	L	5	C/A	R	B
Invasive and other Problematic Species and Genes	-	Dreissenid mussels, Round Goby	K	L	5	H/C/A	C	N

b) Sydenham River

IUCN Threat Category	Sub-category	Details	Likelihood of Occurrence	Level of Impact	Causal Certainty	Threat Occurrence	Threat Frequency	Threat Extent
Pollution	Agricultural and Forestry Effluents	Sedimentation (field runoff, upstream drain maintenance)	K	L	5	H/C/A	C	B
		Nutrient Loading (+ ammonia)	K	L	5	H/C/A	C	B
		Pesticides (+ granular Bayluscide)	K	L	5	H/C/A	C	B
	Domestic and Urban Wastewater (incl. urban runoff)	Nutrient Loading (+ ammonia)	K	L	5	H/C/A	C	B
		Pharmaceuticals and estrogenic compounds	K	L	5	H/C/A	C	B
		Chloride	K	L	5	H/C/A	R	B
		Heavy Metals	K	L	5	H/C/A	C	B
Climate Change and Severe Weather	-	Frequent and severe droughts and heat waves	K	L	5	C/A	R	B
Invasive and other Problematic Species and Genes	-	Dreissenid mussels, Round Goby	K	L	5	H/C/A	C	N

c) Thames River

IUCN Threat Category	Sub-category	Details	Likelihood of Occurrence	Level of Impact	Causal Certainty	Threat Occurrence	Threat Frequency	Threat Extent
Pollution	Agricultural and Forestry Effluents	Sedimentation (field runoff, upstream drain maintenance)	K	L	5	H/C/A	C	B
		Nutrient Loading (+ ammonia)	K	L	5	H/C/A	C	B
		Pesticides (+ granular Bayluscide)	K	L	5	H/C/A	C	B
	Domestic and Urban Wastewater (incl. urban runoff)	Nutrient Loading (+ ammonia)	K	L	5	H/C/A	C	B
		Pharmaceuticals and estrogenic compounds	K	L	5	H/C/A	C	B
		Chloride	K	L	5	H/C/A	R	B
		Heavy Metals	K	L	5	H/C/A	C	B
Climate Change and Severe Weather	-	Frequent and severe droughts and heat waves	K	L	5	C/A	R	B
Invasive and other Problematic Species and Genes	-	Dreissenid mussels, Round Goby	K	L	5	H/C/A	C	N

d) Rationale

Threat Category	Rationale for Scoring
Pollution	Level of Impact: Purple Wartyback populations are currently stable (Ausable River) or growing (Sydenham and Thames rivers), thus, both sub-categories of pollution appear to have a low level of impact. Pollution inputs have the potential for locally extreme impacts in some places (e.g., immediately downstream of point sources), to some life stages (e.g., glochidia), or at certain times of the year (e.g., spring melt), but these are not causing population-level effects at this time. Glochidia are the most sensitive life stage to pollutants like chloride, ammonia, potassium, etc., but Purple Wartyback populations are most sensitive to harm to adults (van der Lee and Koops 2023). Impacts of Domestic and Urban Wastewater (including urban runoff) are likely higher in the Thames River compared to the Ausable or Sydenham rivers due to greater urban density, but still low overall. Causal Certainty: Although a substantial body of literature exists on pollutant impacts to freshwater mussels at both individual- and population-levels, there is no evidence of decline of Purple Wartyback in any of the three populations assessed; the causal certainty was left with high uncertainty (5) as the link between the threat impact and a decline could not be made with currently available information. Threat Frequency (chloride): although chloride levels continuously exceed pre-development conditions in all three waterbodies, pulses of chloride that may be harmful recur periodically.
Climate Change and Severe Weather	Likelihood of Occurrence and Level of Impact: Impacts of climate change (including increased frequency and intensity of droughts and heat waves) have been reported in Ontario compared to historical levels, but impacts to Purple Wartyback have not yet been observed and are likely to be low over the 10 year timeframe evaluated here.
Invasive and other Problematic Species and Genes	Level of Impact: Although the presumed cause of extirpation of Purple Wartyback from the Detroit River and western Lake Erie, dreissenid mussels are less successful in lotic systems (compared to lentic) and are thought to pose a relatively low risk to Purple Wartyback in extant habitats. The impact may be higher in the Thames River immediately downstream of Fanshawe reservoir due to potential for high veliger loads, but veligers are unlikely to be retained at a level that impacts Purple Wartyback. Impacts from Round Goby are likely indirect through host interactions, but these are theoretical as declines in presumed host catfishes have not been documented.

Table 8. The Threat Level Matrix combines the Likelihood of Occurrence and Level of Impact rankings to establish the Threat Level for each Purple Wartyback population in Canada. The resulting Threat Level has been categorized as low, medium, high, or unknown. Reproduced from DFO (2014).

		Level of Impact				
		Low	Medium	High	Extreme	Unknown
Likelihood of Occurrence	Known or very likely	Low	Medium	High	High	Unknown
	Likely	Low	Medium	High	High	Unknown
	Unlikely	Low	Medium	Medium	Medium	Unknown
	Remote	Low	Low	Low	Low	Unknown
	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown

Table 9. Threat Level assessment of each Purple Wartyback population in Canada, resulting from an analysis of both the Threat Likelihood and Threat Impact. The number in brackets refers to the Causal Certainty associated with the threat impact (1 = Very High; 2 = High; 3 = Medium (Med); 4 = Low; 5 = Very Low).

IUCN Threat Category	Sub-category	Details	Ausable River	Sydenham River	Thames River
Pollution	Agricultural and Forestry Effluents	Sedimentation (field runoff, upstream drain maintenance)	Low (5)	Low (5)	Low (5)
		Nutrient Loading (+ ammonia)	Low (5)	Low (5)	Low (5)
		Pesticides (+ granular Bayluscide)	Low (5)	Low (5)	Low (5)
	Domestic and Urban Wastewater (incl. urban runoff)	Nutrient Loading (+ ammonia)	Low (5)	Low (5)	Low (5)
		Pharmaceuticals and estrogenic compounds	Low (5)	Low (5)	Low (5)
		Chloride	Low (5)	Low (5)	Low (5)
		Heavy Metals	Low (5)	Low (5)	Low (5)
Climate Change and Severe Weather	-	Frequent and severe droughts and heat waves	Low (5)	Low (5)	Low (5)
Invasive and other Problematic Species and Genes	-	Dreissenid mussels, Round Goby	Low (5)	Low (5)	Low (5)

Table 10. Species-level Threat Assessment for Purple Wartyback in Canada, resulting from a roll-up of the population-level Threat Assessment. The species-level Threat Assessment retains the highest level of risk for any population, all categories of Threat Occurrence and Threat Frequency are retained, and the species-level Threat Extent is the mode of the population-level Threat Extent.

IUCN Threat Category	Sub-category	Details	Species-level Threat (certainty)	Species-level Occurrence	Species-level Frequency	Species-level Extent
Pollution	Agricultural and Forestry Effluents	Sedimentation (field runoff, upstream drain maintenance)	Low (5)	H/C/A	C	B
		Nutrient Loading (+ ammonia)	Low (5)	H/C/A	C	B
		Pesticides (+ granular Bayluscide)	Low (5)	H/C/A	C	B
	Domestic and Urban Wastewater (incl. urban runoff)	Nutrient Loading (+ ammonia)	Low (5)	H/C/A	C	B
		Pharmaceuticals and estrogenic compounds	Low (5)	H/C/A	C	B
		Chloride	Low (5)	H/C/A	R	B
		Heavy Metals	Low (5)	H/C/A	C	B
Climate Change and Severe Weather	-	Frequent and severe droughts and heat waves	Low (5)	C/A	R	B
Invasive and other Problematic Species and Genes	-	Dreissenid mussels, Round Goby	Low (5)	H/C/A	C	N

Element 9: *Identify the activities most likely to threaten (i.e., damage or destroy) the habitat properties identified in elements 4-5 and provide information on the extent and consequences of these activities.*

Medium to large rivers with moderate to swift currents over substrates of sand, gravel, and cobble capable of supporting larger-bodied ictalurid catfish hosts are the most important habitat features for Purple Wartyback. There are several activities that take place in Purple Wartyback habitat that are likely to damage or destroy these properties, directly or indirectly.

- Agriculture or urban land use practices that result in reduced pervious surfaces and riparian buffers surrounding waterbodies. This can lead to increased sedimentation of watercourses (through bank erosion and run-off) and high water temperatures.
- Dredging for boating infrastructure (e.g., canals, marinas, docks) or for agricultural drainage issues (upstream of Purple Wartyback). This may bury or displace mussels, and can indirectly affect them by altering substrate composition and increasing turbidity. Agricultural drain maintenance is likely to cause resuspension of sediments and nutrients. It may also alter water velocity/shear stress at the substrate surface where mussels are found.
- Bridge and culvert construction or maintenance activities that involve instream works. This is likely to alter substrate at the site, and may impact water velocity and sediment transport leading to increased turbidity.
- Watercourse alterations such as enclosing drains, tiled drains, or channelization. This can result in a loss of habitat, changes in substrate composition, and altered flow and sediment transport.

Element 10: *Assess any natural factors that will limit the survival and recovery of the Purple Wartyback.*

Purple Wartyback, like all unionid mussels, is an obligate parasite at the larval stage requiring a period of encystment on a host fish to complete its life cycle. In Canada, host fishes are thought to be Channel Catfish, Black Bullhead, Yellow Bullhead, and possibly Flathead Catfish. With the exception of Flathead Catfish known only from the lower Thames River, these species are generally common in riverine habitats in Ontario. Purple Wartyback is at risk of predation at all life stages from a number of fish species (e.g., Lake Sturgeon *Acipenser fulvescens*, Freshwater Drum *Aplodinotus grunniens*, Pumpkinseed *Lepomis gibbosus*, redhorses *Moxostoma* spp., catfishes), birds (e.g., diving ducks), and mammals (e.g., mink/fisher, muskrat, raccoon) (Custer and Custer 1996, Mulcrone 2005). Owen et al. (2011) found Purple Wartyback in muskrat middens, but when analyzing electivity of available mussel species, found that Purple Wartyback was generally avoided. Individuals that measured approximately 40–80 mm in length would be most susceptible to predation. Most encounters with predators are likely opportunistic and unlikely to limit Purple Wartyback populations.

Element 11: *Discuss the potential ecological impacts of the threats identified in element 8 to the target species and other co-occurring species. List the possible benefits and disadvantages to the target species and other co-occurring species that may occur if the threats are abated. Identify existing monitoring efforts for the target species and other co-occurring species associated with each of the threats, and identify any knowledge gaps.*

Reduced habitat quality through agricultural and urban land use practices that result in heavy nutrient loads, siltation/sedimentation of watercourses, contaminant inputs from road or field run-off or wastewater, and reduced riparian areas is the biggest threat to Purple Wartyback in Canada. Nutrient loading can result in increased algal growth and decreased dissolved oxygen, which can negatively impact productivity of fishes and mussels. Increased sedimentation can clog siphons, decreasing feeding and respiration, and turbidity may reduce visibility of displaying female mussels or conglutinates leading to reduced host encounters and recruitment success. Mussels may experience toxic impacts from acute or chronic exposure to contaminants that can negatively affect growth, reproduction, and survival. Climate change is likely to have wide ranging impacts that will affect species differently, but generally will exacerbate habitat degradation from anthropogenic disturbances.

Unionids are all sensitive to water quality, and thus any efforts to reduce pollution inputs or sedimentation from agricultural and urban sources would benefit all mussel species co-occurring with Purple Wartyback. Purple Wartyback occurs with many other SARA-listed mussels, particularly in the Sydenham River, which contains 34 mussel species, 14 of which are at risk. The Ausable and Thames rivers also have high mussel diversity with 26 (7 SAR) and 33 (10 SAR) species, respectively (McNichols-O'Rourke et al. 2012). Improved agricultural practices and use of appropriate mitigations (e.g., sediment screens, adequate riparian buffers, cattle fencing), as well as improvements to wastewater treatment plants that input into these rivers (Nikel et al. 2023) would benefit Purple Wartyback and all aquatic species occupying its habitat.

Monitoring for mussel species and their hosts occurs periodically in the three rivers known to contain Purple Wartyback through DFO's Unionid Monitoring and Biodiversity Observation (UMBO) network in partnership with the Ausable-Bayfield Conservation Authority and the St. Clair Region Conservation Authority. As Purple Wartyback is an especially long-lived species, long-term monitoring data is required to properly evaluate impacts of threats or mitigation measures and threat abatement. Water quality monitoring also occurs in those rivers for different purposes. The Provincial Water Quality Monitoring Network samples numerous sites (on a rotating basis) annually on the Ausable, Sydenham and Thames rivers and measures nutrients (total and dissolved), metals, and chloride (Appendix C; MECP 2022). Additional water quality monitoring aimed largely at nutrient management (including total phosphorous, *E. coli*, and benthic invertebrate biomonitoring) is conducted by Conservation Authorities where Purple Wartyback occurs (Appendix D; ABCA 2018a,b, SCRCA 2018, UTRCA 2017a,b,c,d). Monitoring contaminants of concern through time where Purple Wartyback is found would be helpful to understand levels of exposure. A variety of fisheries surveys, notably for SARA-listed fishes periodically and invasive Asian carps annually (e.g., Barnucz et al. 2020, Barnucz and Drake 2021a,b, Aguiar et al. 2021), are conducted in the Ausable, Sydenham, and Thames rivers through the range of Purple Wartyback that could provide an indication of host fish population status and trends, and would likely detect invasive fishes and possibly other aquatic invasive taxa, should they occur.

SCENARIOS FOR MITIGATION OF THREATS AND ALTERNATIVES TO ACTIVITIES

Element 16: *Develop an inventory of feasible mitigation measures and reasonable alternatives to the activities that are threats to the species and its habitat (as identified in elements 8 and 10).*

Threats to species survival and recovery can be reduced by implementing mitigation measures to reduce or eliminate potential harmful effects that could result from works, undertakings, or activities (w/u/a) associated with projects in Purple Wartyback habitat.

Within Purple Wartyback habitat, a variety of w/u/a have occurred in the last several years. The DFO Program Activity Tracking for Habitat (PATH) database was reviewed to estimate the number of w/u/a that have occurred during the period from November 2013 through January 2022 within the known distribution of Purple Wartyback. Twenty-five w/u/a were identified, 24 of which occurred in the Thames River watershed, but these likely do not represent a complete list, as some w/u/a may occur in proximity to Purple Wartyback occurrence records (but outside of the searched distribution) that may also have impacts; and, some w/u/a may not have been reported to DFO as they may have met self-assessment requirements. The review did not include areas with historical records where the species is thought to be extirpated (e.g., Detroit River, Lake Erie at Pelee Island). Project types in the PATH database included: bridge and culvert construction and maintenance (n=8), stream bank stabilization (n=5), dredging (n=3), docks/boathouses (n=3), directional drill piping (n=2), channel modifications (n=1), and stormwater management (n=1). There were no projects authorized under the *Fisheries Act* as most projects were deemed low risk to fish and fish habitat (mussels included) and were addressed through letters of advice with standard mitigations. Without appropriate mitigations, projects or activities occurring adjacent or close to these areas could have impacted Purple Wartyback (e.g., through direct mortality or other physiological impacts, increased turbidity, sedimentation). Based on the assumption that historical and anticipated development pressures are likely to be similar, it is expected that similar types of w/u/a will likely occur in or near Purple Wartyback habitat in the future.

Numerous threats affecting Purple Wartyback populations in Canada are related to habitat loss, degradation or fragmentation. Habitat-related threats to Purple Wartyback have been linked to the Pathways of Effects developed by the Fish and Fish Habitat Protection Program (FFHPP; Table 11). DFO FFHPP has developed guidance on mitigation measures for 18 Pathways of Effects for the protection of aquatic species at risk in the Ontario and Prairie Region (formerly part of Central and Arctic Region) (Coker et al. 2010). This guidance should be referred to when considering mitigation and alternative strategies for habitat-related threats.

In addition to the Pathways of Effects guidance, DFO has developed Codes of Practice for common project types in and around water, including for clear span bridges and culvert maintenance, which should be consulted when these activities occur within the habitat of Purple Wartyback. Similarly, the Ontario Ministry of Agriculture, Food, and Rural Affairs has a number of Best Management Practices relevant for reducing sedimentation, nutrient loads, and other agricultural pollution sources around aquatic environments. Advice has also been developed by DFO for relocating mussels during instream works (Mackie et al. 2008). This advice has been summarized below. Additional mitigation and alternative measures for non-habitat related threats (e.g., invasive and other problematic species and genes) are also provided.

*Table 11. Summary of works, undertakings and activities that have occurred during the period of November 2013 to January 2022 in areas known to be occupied by Purple Wartyback. Threats known to be associated with these types of works, undertakings, and activities are indicated with a checkmark. The number of works, undertakings, and activities associated with each Purple Wartyback population, as determined from the project assessment analysis, has been provided. Applicable Pathways of Effects are indicated for each threat associated with a work, undertaking, or activity: 1 – Vegetation Clearing; 2 – Grading; 3 – Excavation; 4 – Use of explosives; 5 – Use of industrial equipment; 6 – Cleaning or maintenance of bridges or other structures; 7 – Riparian planting; 8 – Streamside livestock grazing; 9 – Marine seismic surveys; 10 – Placement of material or structures in water; 11 – Dredging; 12 – Water extraction; 13 – Organic debris management; 14 – Wastewater management; 15 – Addition or removal of aquatic vegetation; 16 – Change in timing, duration and frequency of flow; 17 – Fish-passage issues; 18 – Structure removal. *contaminants and toxic substances come from agricultural pesticides, and domestic and urban wastewater and runoff.*

Work/Undertaking/Activity	**Threats** (associated with work/undertaking/activity)			**Watercourse/Waterbody** (number of works/undertakings/activities between November 2013 and January 2022)		
	Pollution: Sedimentation	Pollution: Nutrient loading	Pollution: Contaminants and Toxic Substances*	Ausable River	Sydenham River	Thames River
Applicable pathways of effects for threat mitigation and project alternatives	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 15, 16, 18	1, 4, 7, 8, 11, 12, 13, 14, 15, 16	1, 4, 5, 6, 7, 11, 12, 13, 14, 15, 16, 18			
Water Crossings (bridges, culverts, piping)	✓	-	✓	0	1	9
Shoreline/Streambank Works (dykes, bank stabilization, infilling, beach creation, riparian vegetation management)	✓	✓	✓	0	0	5
Dam/Barrier Structures in Water (maintenance, modifications, hydro retrofits)	✓	-	✓	0	0	4
Instream Works (drain maintenance, aquatic vegetation removal, dredging, channel modifications/realignments)	✓	✓	✓	0	0	1
Water Management (stormwater management, water withdrawal)	✓	✓	✓	0	0	0

Work/Undertaking/Activity	**Threats** (associated with work/undertaking/activity)			**Watercourse/Waterbody** (number of works/undertakings/activities between November 2013 and January 2022)		
Applicable pathways of effects for threat mitigation and project alternatives	Pollution: Sedimentation	Pollution: Nutrient loading	Pollution: Contaminants and Toxic Substances*	Ausable River	Sydenham River	Thames River
	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 15, 16, 18	1, 4, 7, 8, 11, 12, 13, 14, 15, 16	1, 4, 5, 6, 7, 11, 12, 13, 14, 15, 16, 18			
Structures in Water (boat launches, docks/boathouses, effluent outfalls, water intakes)	✓	✓	✓	0	0	4

MUSSEL RELOCATION PROTOCOL

Guidance for conducting surveys to detect the presence of SAR mussels, relocating mussels during w/u/a, and conducting post-relocation monitoring is provided in Mackie et al. (2008). This guidance is intended for projects planned in and around water, such as bridge or culvert construction, pipeline crossings, and dredging activities where SAR mussels may be affected. After determining that SAR mussels are present, that a relocation is deemed feasible, and appropriate permits have been obtained, the relocation may begin. See Mackie et al. (2008) for detailed methodology.

Mitigations

- Identify a suitable relocation site, typically upstream of the w/u/a, that has similar habitat properties (area, water depth, substrate types, water velocity), and biotic structure (fish and mussel communities, absence of AIS).
- Conduct relocation at least one month before water temperature is likely to drop below 16 °C (usually mid to late August in Ontario).
- Ensure all juvenile and adult mussels are removed from impacted area.
- Keep mussels moist or in water, avoid overcrowding, and minimize transit time to reduce stress on mussels.
- Aim to replace mussels in the same orientation and in similar substrate as they were found in.
- Conduct follow-up monitoring one month, one year, and two years after the relocation. Monitoring must be conducted when water temperatures are > 16 °C to ensure mussels can rebury themselves.

Alternatives

- If project is planned around a mussel bed or near a high-density patch of SAR mussels, consider relocating project downstream or redesigning the project to avoid instream effects.

INVASIVE AND OTHER PROBLEMATIC SPECIES AND GENES

Several aquatic invasive taxa threaten Purple Wartyback directly (through competition/ predation) and indirectly (through habitat modifications or attachment/biofouling).

Mitigations

- Develop public awareness campaigns and encourage the use of existing invasive species reporting systems (e.g., Ontario Invading Species Awareness Program hotline, EDDMapS).
- Conduct early detection surveillance or monitoring for invasive species that may negatively affect Purple Wartyback populations directly, or negatively affect its habitat.
- Develop a response plan to address potential risks, impacts, and proposed actions if monitoring detects the arrival or establishment of an invasive species.

Alternatives

- Unauthorized introductions
  - None
- Authorized introductions
  - Do not stock non-native species in areas inhabited by Purple Wartyback.
  - Do not enhance habitat for non-native species in areas inhabited by Purple Wartyback
  - Follow the National Code on Introductions and Transfers of Aquatic Organisms for all aquatic organism introductions (DFO 2017).

SOURCES OF UNCERTAINTY

Sources of uncertainty have been organized into research themes based on Drake et al. (2021) to create consistency across RPAs and to aid in planning and prioritization of research objectives.

POPULATION ECOLOGY

Life History

There are several gaps in our knowledge of Purple Wartyback life history. In particular, timing of the spawning season is not known for Ontario. Low numbers of sperm and eggs were observed in Purple Wartyback in Ontario in June through July (suggesting spawning had likely already occurred) (COSEWIC 2021), and glochidia have been detected in late summer (Smodis 2022); however, precise timing is unknown and would be helpful for understanding suitable timing windows for instream projects, and whether pulses of chloride and other contaminants associated with spring melt coincide with presence of pollution-sensitive glochidia. The diet of Purple Wartyback is unknown, as are feeding strategies, particularly of juveniles (i.e., duration of pedal feeding period).

Abundance

Fulsome estimates of abundance are lacking for all Purple Wartyback populations in Canada (current estimates are spatially restricted). Including habitat and density information from a randomized study design would offer a better understanding of how habitat influences density, and allow more accurate projections of population abundance across the river systems.

Distribution

Despite extensive survey effort, there remains uncertainty in the full distribution of Purple Wartyback within known locations, and possibly beyond. The distribution in the Ausable River was considered two distinct stretches of river, but a recent (2019) detection in between these two stretches suggests it could occupy a larger space there. Similarly, on the Thames River, Purple Wartyback is known from Delaware to Kent Bridge, and is assumed to occupy the river down to the mouth; however, due to water depth and turbidity limiting sampling, this is not confirmed. One individual was recently detected in Black Creek, a tributary of the North Sydenham River, but it is unknown whether a viable population exists in this tributary.

Species Interactions

There are many uncertainties surrounding mussel-host interactions. Large-bodied ictalurid catfishes are the presumed host fishes of Purple Wartyback in Canada, but this has not been confirmed. There is no information on optimal density of host fishes for successful encounters, or on infestation or metamorphosis rates of glochidia on hosts. The dispersal ability of glochidia while encysted on host fishes is also unknown (this may help understand the Black Creek detection).

HABITAT

Species-habitat Associations by Life Stage

Purple Wartyback appears relatively tolerant of a range of environmental conditions, but optimal habitat for completing life-history processes remains unknown. Although adults and juveniles have been observed in the same areas, adults typically live at the substrate-water interface, while juveniles are buried beneath the surface. The ideal (or upper limits on) habitat conditions (e.g., flow rate, substrate type, dissolved oxygen, temperature) and food availability in those micro-habitats is unknown.

Habitat Supply

The Purple Wartyback distribution is considered distinct in each of the three occupied rivers, but it is unlikely that the entirety of these stretches contain suitable habitat. As the ideal habitat properties for Purple Wartyback are not explicitly known, the spatial extent of these properties within the (historically and currently) occupied rivers also remains unknown. Additionally, the quantity of habitat needed to support healthy and sufficiently dense populations of host fishes is unknown.

THREATS

Mechanism of Impact

All freshwater mussels are known to be pollution sensitive, but there have been few, if any, toxicology studies on Purple Wartyback specifically to understand lethal limits or broader impacts on life history (e.g., growth, metabolism, reproduction). Toxicity ranges are available from multi-species studies and it is likely that Purple Wartyback falls within these ranges, but understanding of an appropriate surrogate would be beneficial to contextualize Purple Wartyback response within these known toxicity ranges. Climate change will have numerous impacts on aquatic ecosystems, many of which are likely to interact with other anthropogenic stressors. The mechanisms through which climate change will impact Purple Wartyback and its host fishes remain unknown; however, further study of the physiological tolerances to environmental stressors (e.g., temperature, dissolved oxygen, rapid changes in flow) would help resolve some uncertainties.

Probability, Extent, and Magnitude of Impact

Although point measurements of some contaminants are available in the watersheds in which Purple Wartyback is found, environmentally realized concentrations at localities occupied by the species, and the persistence of contaminants in the water column and at the substrate surface are unknown. It is unclear to what extent dams and other barriers may impact the dispersal of host fishes, and to what extent that affects Purple Wartyback dispersal. Furthermore, the dams are relatively new in the context of the Purple Wartyback lifespan for effects to be determined. The mechanisms of impact of AIS are often clear (e.g., competition, predation, habitat alteration, biofouling), but the extent and magnitude of these impacts on Purple Wartyback from Round Goby, and dreissenid mussels in riverine habitats remain unclear.

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APPENDIX A

DFO conducts an ongoing quadrat-based monitoring (Unionid Monitoring and Biodiversity Observation (UMBO)) network for tracking unionid mussels in southern Ontario that includes sampling in the Ausable, Sydenham, and Thames rivers. Van der Lee et al. (in prep.[1]) analyzed UMBO network data to estimate Purple Wartyback density and trajectory in the Sydenham and Thames rivers to improve our understanding of Purple Wartyback population ecology. This analysis was repeated for the Ausable River when data became available. The model included six Ausable River sites from which Purple Wartyback could occur that were sampled three times between 2006 and 2022, representing three sampling periods of the standardized monitoring sites. Survey methods used at each index station during both sampling events were modified from Metcalfe-Smith et al. (2007) and generally follow Sheldon et al. (2020). There were 1,340 quadrat samples included in the model; Purple Wartyback were found in 105 quadrats, and 136 individuals were collected in total. Population density and trajectory were estimated by fitting a model to the Ausable River UMBO data. A hierarchical Bayesian approach was taken using Integrated Nested Laplace Approximation (INLA; Rue et al. 2009). INLA uses deterministic methods to make Bayesian inferences. The model fit was a Poisson regression with site as a random effect and year as the only covariate. From this model we can get an estimate of expected mean density and an estimate of population growth rate. Refer to van der Lee et al. (in prep.[1]) for more detailed methods, and companion results for the Sydenham and Thames rivers.

$$y_{is} \sim Poisson(\mu_{is}) \ ,$$

$$E(y_{is}) = var(y_{is}) = \mu_{is},$$

$$\log(\mu_{is}) = \alpha + \beta_1 \cdot year_i + site_s.$$

Where y_{is} represents the PWB counts from quadrat i and site s and μ_{is} is the expected mean. α is the intercept representing the initial mean density as the covariate $year$ is the year sampled beginning at 0 (survey year subtracted from the first survey year). β is the slope of the year effect representing the instantaneous rate of population growth with population growth rate, $\lambda = e^{\beta}$. $site$ represents the site random effect.

Table A1. Parameter estimates for quadrat-count INLA models for Ausable River Purple Wartyback.

	Median	LCI	UCI	sd
Fixed Effects				
Intercept	-3.64	-5.91	-1.84	1
Year	0.016	-0.015	0.048	0.015
Hyper-parameters				
site	0.25	0.05	0.79	0.21

The estimate of population growth rate was 1.016 (95% CI: 0.985–1.049) but did not differ significantly from 1 indicating that there is no evidence of positive population growth nor a decline over the sample period. Average density estimated for 2022 (the most recent sample year) was low, 0.031 mussels/m^2 (95% CI: 0.002–0.25). Abundance was estimated at the survey sites as 294 (95% CI: 207-409) Purple Wartyback in 2,490 m^2 of habitat. The mean length was 60.3 mm, and juveniles (mussels < 53.15 mm) made up 33.8% of the sampled individuals. There were no significant trends in the mean size or proportion of juveniles in the sample, but the consistent presence of juveniles suggests on-going recruitment. Overall, the results suggest that there is no change in the population size or structure.

APPENDIX B

Table B1. Summary of current (1997–2022) mussel sampling effort within the current range of Purple Wartyback (PWB) in Canada. Several sampling methods are included as well as incidental observations. PH refers to the number of person-hours searched, where available. Data is summarized from the Lower Great Lakes Unionid Database with additional data sources where applicable. Superscript indicates number of sites with shells only. Adapted from COSEWIC (2021). Note that not all 2022 sampling data may be available at this time.

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Ausable River	1998	2^{1}/10	4 (2)	39.75 PH (1.5-4.5 PH per site)	Timed-search surveys	-
Ausable River	1999	0/1	0	No effort recorded	Timed-search survey	-
Ausable River	2001	0/1	0	No effort recorded	Timed-search survey	-
Ausable River	2002	1/4	2	18 PH (4.5 PH per site)	Timed-search surveys	-
Ausable River	2003	0/2	0	No effort recorded	Observational records	-
Ausable River	2004	0^{1}/8	0 (1)	36 PH (4.5 PH per site)	Timed-search surveys	-
Ausable River	2005	0/1	0	No effort recorded	Observational records	-
Ausable River	2006	3/7	38	506 x 1 m^2 quadrats with excavation (7 sites; 69-75 quadrats per site)	Index station surveys by ABCA	Baitz et al. 2008 , Upsdell et al. 2012
Ausable River	2007	1/2	2	66 x 1 m^2 quadrats with excavation (1 site); 2.25 PH	Timed-search surveys	Ausable Bayfield Conservation Authority (ABCA) unpublished data
Ausable River	2008	2^{1}/12	14 (1)	75 x 1 m^2 quadrats with excavation (1 sites; 75 quadrats per site); 18 PH (4.5 PH at four sites)	Index station surveys by ABCA; timed-search surveys by D. Zanatta	ABCA unpublished data

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Ausable River	2009	0/7	0	174 x 1 m^2 quadrats with excavation (2 sites; 87 quadrats at each site); 9 PH (4.5 PH at two sites)	Index station surveys by ABCA; timed-search surveys by ABCA; timed-search survey by D. Zanatta	ABCA unpublished data
Ausable River	2010	0/1	0	No effort recorded	Observational records from ABCA	ABCA unpublished data
Ausable River	2011	2/7	26	534 x 1 m^2 quadrats with excavation (7 sites; 74-80 quadrats per site)	Index station survey by ABCA	Upsdell et al. 2012
Ausable River	2012	1/1	25	No effort recorded	Community behaviour study	
Ausable River	2013	2/3	43	75 x 1 m^2 quadrats with excavation (1 site); 5.0 PH (1 site); other effort unknown	Index station survey by ABCA; timed-search surveys; DFO behaviour study	ABCA unpublished data
Ausable River	2014	1/1	3	No effort recorded	Targeted survey	ABCA unpublished data
Ausable River	2015	1/1	2	No effort recorded	Targeted survey	ABCA unpublished data
Ausable River	2016	1/1	10	No effort recorded	DFO behaviour study	DFO unpublished data
Ausable River	2018	2/4	13	301 x 1 m^2 quadrats with excavation (4 sites; 75–76 quadrats per site); other effort unknown	Index station surveys by ABCA; timed-search surveys	ABCA unpublished data
Ausable River	2019	2/3	27	226 x 1 m^2 quadrats with excavation (3 sites; 75–76 quadrats per site)	-	-

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Ausable River	2022	1/1	15	75 x 1 m^2 quadrats with excavation (1 site; 75 quadrats per site)	-	-
Sydenham River	1997	7/8	241 (54)	36 PH (4.5 PH per site)	Timed-search surveys	-
Sydenham River	1998	4[1]/5	40 (3)	18.5 PH (4.5-5 PH per site)	Timed-search surveys	-
Sydenham River	1999	2/8	44	147 x 1 m^2 quadrats with excavation (2 sites; 69-78 quadrats per site)	Index station surveys; timed-search surveys	Metcalfe-Smith et al. (2007)
Sydenham River	2000	0/1	0	No effort recorded	Observational record	--
Sydenham River	2001	2/18	95	230 x 1 m^2 quadrats with excavation (3 sites; 75-80 quadrats per site)	Index station surveys; timed-search surveys by the University of Guelph	Metcalfe-Smith et al. (2007)
Sydenham River	2002	4/43	704	381 x 1 m^2 quadrats with excavation (5 sites; 72-81 quadrats per site); 4.5 PH (at one site)	Index station surveys; timed-search surveys by the University of Guelph	Metcalfe-Smith et al. (2007)
Sydenham River	2003	3/15	392	387 x 1 m^2 quadrats with excavation (5 sites; 69-84 quadrats per site); 75.67 PH (6-40.67 PH per site)	Index station surveys; timed-search surveys by the University of Guelph	Metcalfe-Smith et al. (2007)
Sydenham River	2004	0/2	0	46 PH (22.67-23.33 PH per site)	Timed-search surveys	-
Sydenham River	2005	0/9	0	40 PH (7.5-20.5 PH per site)	Timed-search surveys by the University of Guelph	-

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Sydenham River	2006	0/6	0	20.5 PH (1.5-19 PH per site)	Timed-search surveys by the University of Guelph	-
Sydenham River	2007	0/2	0	16 PH (1-15 PH per site)	Timed-search surveys	-
Sydenham River	2008	5/19	110	168 m2; 34.52 PH (1.6-10.67 PH per site)	Excavation using a crane mounted clam bucket by G.L. Mackie; Timed-search surveys by D. Zanatta; Timed-search surveys by the University of Guelph	-
Sydenham River	2009	0/14	0	45.97 PH (1.3-12.75 PH per site)	Timed-search surveys by D. Zanatta	-
Sydenham River	2010	2/3	25	37.5 PH (15-22.5 PH per site)	Timed-search surveys; Observational record	-
Sydenham River	2011	0/7	0	102 PH (4.5-32 PH per site)	Timed-search surveys by the University of Guelph	-
Sydenham River	2012	6/12	2886	669 x 1 m^2 quadrats with excavation (5 sites; 69-375 quadrats per site); 235 PH (5-192 PH per site)	Index station surveys; timed-search surveys by the University of Guelph	-
Sydenham River	2013	6/11	981	375 x 1 m^2 quadrats with excavation (5 sites; 75 quadrats at each site); 120.5 PH (9-60 PH per site)	Index station surveys; timed-search surveys by the University of Guelph; Reproductive study by DFO	-
Sydenham River	2014	3/4	153	60 PH (14-25 PH per site)	Timed-search surveys; Reproductive study by DFO	-

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Sydenham River	2015	4/7	424	225 x 1 m^2 quadrats with excavation (3 sites; 75 quadrats at each site); 24 PH (2-14 PH per site)	Index station surveys; timed-search surveys by the University of Guelph	-
Sydenham River	2016	0/5	0	71 PH (20-27 PH per site)	Timed-search surveys; Observational records by SCRCA	-
Sydenham River	2017	8/11	217	50 x 1 m^2 quadrats with excavation (5 sites; 10 quadrats per site); 64.5 PH (4.5-42 PH per site)	Quantitative surveys at the Sydenham River Nature Reserve; timed-search surveys; Ontario Freshwater Mussel Identification Workshop	-
Sydenham River	2018	2/2	29	22 PH (at one site)	Ontario Freshwater Mussel Identification Workshop	-
Sydenham River	2019	5/15	11 (1)	128 PH (16 8-hour airlift bed excavations); 30.95 PH (0.7-4.5 PH per site)	Index station surveys; timed-search surveys	-
Sydenham River	2020	10[1]/13	268 (2)	Combo: 100 x 1 m^2 quadrats with excavation (10 sites; 10 quadrats per site) and 45 PH (9 sites; 4.5 PH per site); 317 m^2 (2 quadrat sites); 4.5 PH (1 site)	Index station surveys; timed-search surveys	-
Sydenham River	2021	11/20	265	80 x 1 m^2 quadrats with excavation (8 sites; 10 quadrats per site); 45 PH (10 sites; 4.5 PH per site)	Index station surveys; timed-search surveys	DFO and SCRCA unpublished data

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Sydenham River	2022	10/13	680	375 x 1 m^2 quadrats with excavations (5 sites; 75 quadrats per site); and 36 PH (8 sites; 4.5 PH per site)	Index station surveys; timed-search surveys	DFO and SCRCA unpublished data
Thames River	1997	6[3]/11	32 (34)	49.5 PH (4.5 PH per site)	Timed-search surveys	Metcalfe-Smith et al. (1998), Metcalfe-Smith et al. (2000)
Thames River	1998	0/9	0	22.5 PH (4.5 PH at five sites)	Timed-search surveys	Metcalfe-Smith et al. (2000)
Thames River	2003	0[2]/11	0 (3)	29 PH (1.0-5.0 PH per site; 11 sites)	Timed-search surveys	UTRCA unpublished data
Thames River	2004	4[2]/21	21 (3)	336 x 1 m^2 quadrats with excavation (5 sites; 63-72 quadrats per site); 72 PH (4.5 PH per site)	Index station surveys; timed-search surveys	Morris and Edwards (2007), DFO unpublished data
Thames River	2005	9/10	65 (1)	69 x 1 m^2 quadrats with excavation (1 site); 40.5 PH (4.5 PH at nine sites)	Index station survey; timed-search surveys	Morris and Edwards (2007), DFO unpublished data
Thames River	2006	0/1	0	No effort recorded	Survey by the University of Guelph	-
Thames River	2008	3/14	20	18 PH (4.5 PH at four sites)	Timed-search surveys; temporal study	-
Thames River	2009	0/2	0	No effort recorded	Vertical movement behaviour study	-
Thames River	2010	2/8	7	318 x 1 m^2 quadrats with excavation (5 sites; 15-78 quadrats per site); 1 PH (at one site)	Index station survey; timed-search survey by the University of Guelph; incidental observation	DFO unpublished data

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Thames River	2011	0/12	0	1,069 x 1 m^2 quadrats with excavation (30-999 quadrats per site); 75 PH (1-18 PH per site)	Timed-search surveys with excavation; incidental observation	-
Thames River	2012	2/9	39	696 x 1 m^2 quadrats with excavation (10-318 quadrats per site)	Quantitative surveys	-
Thames River	2013	1[1]/11	37 (1)	636 x 1 m^2 quadrats with excavation (318 quadrats per site); 70 PH (1-33 PH per site)	Relocation with excavation; timed-search surveys	-
Thames River	2014	0/4	0	84 PH (14-30 PH per site)	Timed-search surveys	-
Thames River	2015	3/7	30 (1)	300 x 1 m^2 quadrats with excavation (4 sites; 69-75 quadrats per site; 45.5 PH (12-17.5 PH per site)	Index station surveys; timed-search surveys	DFO unpublished data
Thames River	2016	2/10	125	375 x 1 m^2 quadrats with excavation (5 sites; 75 quadrats excavated at all five sites); 38 PH (4-10 PH per site)	Index station surveys; timed-search surveys	DFO unpublished data
Thames River	2017	2/4	9	225 x 1 m^2 quadrats with excavation (3 sites; 75 quadrats per site)	Index station surveys; timed-search surveys	DFO unpublished data
Thames River	2018	2/7	15	300 x 1 m^2 quadrats with excavation (4 sites; 75 quadrats per site); 6 PH (2.5-3.5 PH)	Index station surveys; timed-search survey; LTVCA timed-search survey; UTRCA timed-search survey	Sheldon et al. (2020)

Waterbody	Year	# of sites w live PWB/total # of sites surveyed	# of live PWB (# shells)	Total Effort	Notes	Other Sources
Thames River	2021	5[1]/11	42 (1)	37.5 PH (9 sites; 3.0-4.5 PH per site); 2 sites targeted	Index station surveys; timed-search surveys	DFO unpublished data
Thames River	2022	14[2]/24	102 (2)	108 PH (24 sites; 4.5 PH per site)	timed-search surveys	DFO unpublished data

APPENDIX C

Table C1. Water quality monitoring conducted by conservation authorities for watershed report cards (ABCA 2018a,b; SCRCA 2018; UTRCA 2017a,b,c,d). Sub-watersheds that cover the known distribution of Purple Wartyback in each river are reported. Orange indicates the values exceed the upper objective set by Conservation Ontario. Upper objective for Total Phosphorous is 0.03 mg/L (Provincial Water Quality Monitoring Objective), for E. coli *is 100 Colony Forming Units CFU)/100 Ml (Ontario Ministry of Environment, Conservation and Parks standards for safe swimming), and for benthic invertebrate sampling is 5.0.*

Population	Subwatershed (PWB Distribution)	Total Phosphorous (mg/L)			Bacteria (E. Coli CFU/100Ml)			Benthic Score		
		2001-2005	2006-2010	2011-2015	2001-2005	2006-2010	2011-2015	2001-2005	2006-2010	2011-2015
Ausable River	Middle Ausable River*	-	0.094	0.076	-	70	92	-	4.47	4.51
	Lower Ausable River*	-	0.067	0.091	-	42	71	-	5.71	5.99
Sydenham River	Upper Sydenham River	0.090	0.080	0.110	155	223	308	5.9	6.01	5.31
	Middle East Sydenham	0.080	0.080	0.120	99	162	234	5.76	5.55	4.88
	Lower East Sydenham	0.060	0.080	0.090	86	50	80	5.48	5.53	5.45
Thames River	Plover Mills - North Thames River	0.077	0.087	0.114	99	35	80	5.66	5.6	4.99
	Dorchester - South Thames River	0.170	0.170	0.100	203	250	202	6.53	6.08	5.83
	the Forks - Thames River	0.220	0.190	0.150	396	617	404	6.38	6.17	6.36
	River Bend Watershed - Thames River	0.140	0.135	0.163	143	202	245	6.19	5.9	6.22

*sample years were 2012 and 2017

APPENDIX D

Table D1. Mean and maximum (max) Provincial Water Quality Monitoring Network values summarized across sampling stations in the Ausable, Sydenham and Thames rivers collected from 2016 through 2020. Accessed from: Provincial (Stream) Water Quality Monitoring Network [March 8, 2022].

Parameter	Units	Ausable River		Sydenham River		Thames River		North Thames River	
		Mean	Max	Mean	Max	Mean	Max	Mean	Max
Alkalinity (Total)	mg/L	197.95	286	207.24	623	186.61	279	181.40	281
Aluminium (Unfiltered Total)	µg/L	118.67	922	215.12	1400	350.78	2300	171.47	1260
Ammonium (Total Unfiltered Reactive)	mg/L	0.09	1.7	0.06	0.3	0.11	0.851	0.12	0.864
Barium (Unfiltered Total)	µg/L	35.76	49.6	20.26	49.2	42.15	118	31.54	46.2
Berylium (Unfiltered Total)	µg/L	0.08	0.235	0.08	0.25	0.09	0.4	0.06	0.2
Bismuth (Unfiltered Total)	µg/L	1.38	2.75	1.09	2.25	1.42	5.17	0.61	1.21
Cadmium (Unfiltered Total)	µg/L	0.60	1.68	0.56	1.55	0.62	1.62	0.63	2.17
Calcium (Unfiltered Total)	mg/L	65.70	110	61.92	87.8	77.58	107	69.80	93.8
Carbon (Dissolved Inorganic)	mg/L	48.41	66.5	40.31	67.5	46.91	65.2	49.01	65
Carbon (Dissolved Organic)	mg/L	3.24	6.3	5.14	10.3	4.52	8.1	4.60	8.8
Chloride (Unfiltered Reactive)	mg/L	34.86	95.9	32.70	66.4	81.63	200	61.53	272
Chromium (Unfiltered Total)	µg/L	0.53	1.27	0.68	3.17	0.88	3.68	0.61	1.69
Cobalt (Unfiltered Total)	µg/L	0.46	1.32	0.55	1.5	0.84	3.15	0.62	1.17
Conductivity (25°C)	µs/cm	563.11	809	578.53	776	743.84	1290	657.92	1900
Copper (Unfiltered Total)	µg/L	1.96	7.77	2.09	11.6	3.18	9	3.23	6.69
Hardness (Total)	mg/L	234.92	351	234.06	328	272.64	367	264.46	341
Iron (Unfiltered Total)	µg/L	123.09	579	287.50	1410	518.48	3870	146.85	689
Lead (Unfiltered Total)	µg/L	0.40	0.7	1.06	1.57	1.70	7.08	4.46	4.46
Lithium (Unfiltered Total)	µg/L	3.44	9.48	5.02	23.2	6.86	20.3	6.19	18.9
Magnesium (Unfiltered Total)	mg/L	16.36	21.9	19.75	30.6	19.22	26.1	22.35	27.4
Manganese (Unfiltered Total)	µg/L	18.28	49.8	24.30	75.6	48.84	202	24.24	107
Molybdenum (Unfiltered Total)	µg/L	1.01	3.45	1.11	3.42	1.59	3.71	1.90	7.92
Nickel (Unfiltered Total)	µg/L	0.71	2	0.87	3.75	1.89	12.4	1.04	2.83
Nitrates (Total Unfiltered Reactive)	mg/L	4.59	10.6	3.66	17.1	5.50	12.8	6.79	36.1
Nitrite (Unfiltered Reactive)	mg/L	0.02	0.075	0.04	0.49	0.05	0.31	0.04	0.19
Nitrogen (Total)	mg/L	4.98	12.7	4.03	21.2	7.10	211	7.68	38.6
pH (Field)		8.24	10.27	8.08	12.6	8.13	9.84	8.47	10.49
Phosphate (Filtered Reactive)	mg/L	0.04	0.191	0.04	0.867	0.05	0.43	0.05	0.297
Phosphorus (Unfiltered Total)	µg/L	82.92	508	84.03	532	131.97	865	109.11	654
Phosphorus (Unfiltered Total)	mg/L	0.08	0.335	0.05	0.172	0.09	0.26	0.08	0.372
Potassium (Unfiltered Total)	mg/L	3.14	8.93	2.06	5.64	4.42	10.1	6.66	24.2
Silicates (Unfiltered Reactive)	mg/L	1.17	3.04	2.29	4.4	1.99	4.36	1.59	4
Silver (Unfiltered Total)	µg/L	0.93	2.59	0.83	2.72	1.03	3.62	1.58	6.08

Parameter	Units	Ausable River		Sydenham River		Thames River		North Thames River	
		Mean	Max	Mean	Max	Mean	Max	Mean	Max
Sodium (Unfiltered Total)	mg/L	21.81	59.4	10.35	19.7	51.19	134	66.51	265
Strontium (Unfiltered Total)	µg/L	309.24	924	118.85	217	502.07	931	3707.9	19000
Tin (Unfiltered Total)	µg/L	2.13	4.46	0.86	1.39	1.73	4.23	4.34	7.48
Titanium (Unfiltered Total)	µg/L	3.75	28.3	1.78	21.8	4.93	31.1	2.86	14.3
Turbidity (Field)	FNU	18.96	79.2	23.31	155.1	32.43	382.9	19.79	175.4
Uranium (Unfiltered Total)	µg/L	5.19	12.5	5.62	9.43	5.70	11.2	7.21	25.6
Vanadium (Unfiltered Total)	µg/L	0.67	1.71	0.74	2.45	1.29	5.66	1.46	3.19
Zinc (Unfiltered Total)	µg/L	10.02	17.8	14.17	97.1	15.05	56	14.77	38
Zirconium (Unfiltered Total)	µg/L	0.23	0.71	0.21	0.79	0.16	0.7	0.19	0.65